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CANCER RESEARCH

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CANCER RESEARCH

A MONTHLY JOURNAL OF ARTICLES AND ABSTRACTS REPORTING CANCER RESEARCH

VOLUME 5 NOVEMBER, 1945 NUMBER 11

The Dependence of Tumor Formation on the Degree of Caloric Restriction*

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(Received for publication June 20, 1945)

In previous publications (4, 5) it has been reported that caloric restriction of the diet causes an inhibition of tumor formation in the mouse. This effect occurred whether the restriction was obtained by decreasing only the carbohydrate component of the diet (so-called caloric restriction *per se*) or by feeding smaller amounts of the *ad libitum* ration (all components restricted proportionately).

In our laboratory, the inhibitory effect of caloric restriction has been demonstrated for spontaneous mammary and lung tumors and for induced epithelioma and sarcoma. Since caloric restriction affected these four different types in a similar manner it was pointed out that some general influence must be involved: Earlier McCay and his associates (2) had indicated that tumors of the rat are similarly inhibited. Other workers have extended the observations on mammary tumors (8, 9), and the inhibitory effect of caloric restriction has been demonstrated also for induced and spontaneous leukemias (3, 9).

In all this work the control group was compared with a restricted group whose caloric intake was approximately 60 per cent or less of the *ad libitum* intake. This level of restriction was chosen by us because of practical considerations; it is approximately the level below which, with ordinary care, it is difficult to maintain a group of mice so that they live at least the average life span. In fact, as has been shown in previous work (4, 5), mice restricted at such a level actually live longer on the average than even those control mice that do not develop tumors.

For some years we have been carrying on a series of experiments in which more than one restricted group was employed; here the restricted groups differed in the degree of restriction. The first of these experiments (graded restriction of the ration) was reported in an earlier publication (4, page 339). In the present experiments, on spontaneous mammary and induced skin tumors, the effect of varying degrees of caloric restriction was studied. The restricted diets, which differed from one another and from the *ad libitum* diet only in the amount of carbohydrate, ranged from approximately 60 to 90 per cent of the *ad libitum* diet in caloric value.

It seemed worth while to learn the effects of various degrees of caloric restriction, because (a) such information might be of assistance in interpreting the results of many nondietary experimental studies (in cancer research), in which the experimental procedure might result in an altered food intake or assimilation in comparison with the control group; and (b) some information might be gained concerning the mechanism of the caloric effect.

EXPERIMENTAL

In all the experiments pure strain mice, bred in our laboratory, were used. Those in any one experiment were born within a span of a few weeks and divided into groups equivalent as to age, weight, and sex. As far as possible, litter mates were distributed between the groups. They were weaned at about 1 month, and were fed Purina fox chow checkers until the respective experimental diets were initiated. Each animal was numbered and a separate record of its weight and progress was kept.

Except where noted, each group of 5 mice was kept in 2 cages; at the biweekly weighings the lighter animals were placed in one cage, the heavier in the other. Thus the animals competed with others of the same order of weight and, over the long period of the experiment, consumed approximately equal quantities of food.

At 2-week intervals the animals were inspected for neoplasms and weighed. The mice were examined post mortem when the tumors became large at death, or at the termination of the experiment. Percentages of

* This investigation was aided by a grant from the National Cancer Institute, Bethesda 14, Maryland.

tumors were computed on the basis of the number of animals alive in the group at the time the first tumor appeared in the experiment (effectual total).

The diets were formulated with two objectives: (a) that the control diet be adequate for health and growth, and (b) that the restricted diets be restricted in calories (carbohydrate) only, but contain the same amount of noncarbohydrate components (proteins, fats, vitamins, and minerals) as the control diet. This was accomplished by feeding the restricted groups and the *ad libitum* group of the series the same amount of Purina fox chow meal and skimmed milk powder, and adding different amounts of cornstarch to the several diets. The details are given under the respective experiments. The compositions and caloric values of the diets were calculated from the data supplied by the manufacturers of the foodstuffs.

A week's supply of the weighed dietary constituents was mixed with sufficient water to form an easily molded mash, cut into equal blocks, and stored in a refrigerator. The mice were fed daily. Those on the restricted diets consumed all the food given them. The actual food consumption of the control group was estimated by weighing back each week the food left in the cages. All animals had free access to water.

Experiment 1.—The 3 experimental groups were each composed of 50 virgin dba females, all born between May 27 and June 6, 1940. The mice were transferred to their respective experimental diets when they were 9 to 11 weeks of age. The mice of group N42 were housed 5 to a cage, while the other 2 groups were housed as described above. Group N42 was fed *ad libitum,* N44 given 2.3 gm. of food per mouse daily, and N45 2.0 gm. per mouse daily. The composition of the diet, mean caloric intake, and mean weight of the mice are given in Table Ia.

The experiment was continued until the mice were 100 weeks old. Throughout the experimental period the mice of all groups were in relatively good health. As can be seen in Fig. 1, only 2 deaths occurred (out of an effectual total of 147 mice) by the time the mice were 1 year old. The number of animals alive at the end of the experiment, and the mean age at which death occurred from nontumor causes (Table Ib), indicate that the experiment ran smoothly.

The data with regard to tumor formation and the average time of appearance of the tumors are listed in Table Ib. The graphs showing cumulative tumor formation are given in Fig. 1. By the end of the experiment the 3 groups N42, N44, and N45, consuming 10 to 12, 8, and 7 calories respectively, had developed 54, 12, and 0 per cent mammary tumors. It therefore appears that the degree of caloric restriction is a factor in determining the degree of inhibition of tumor formation.

Experiment 2.—The 5 experimental groups of this series were each composed of either 25 or 30 parous dba females, all born between December 16, 1940 and January 16, 1941. The respective experimental diets were instituted when the mice were 21 to 25 weeks of age.

All the experimental diets contained 1.4 gm. of fox chow meal and 0.9 gm. of skimmed milk powder per mouse per day. Cornstarch was added in graded amounts so that the diets varied from an *ad libitum* ration of 3.3 gm. per day to the most restricted diet,

Table Ia: Diet, Mean Caloric Intake, and Mean Weight of Mice of Experiment 1

Group	Diet constituents (gm. per mouse per day)				Mean daily caloric intake	Percentage composition of diets				Mean weight of mice in gm.					
										Age in weeks					
	Fox chow meal	Skimmed milk powder	Corn-starch	Total		Protein	Fat	Carbo-hydrate	Ash	10	21	41	61	80	General † level
N42	1.2	0.8	1.4	3.4	10-12 *	17	2	67	4	20	25	29	31	29	30
N44	1.2	0.8	0.3	2.3	8.2	25	3	57	6	20	21	22	24	20	22
N45	1.2	0.8		2.0	7.1	29	3	52	7	21	18	21	20	20	20

* This caloric intake was computed from actual food consumption, which varied during the course of the experiment.
† Approximate mean weight between 40 and 90 weeks of age.

Table Ib: Effect of Caloric Intake on the Formation of Spontaneous Mammary Tumors in dba Virgin Female Mice

Group	Mean caloric intake	Number of mice (effectual total) *	Mice developing breast tumors by 100 weeks of age (end of experiment)			Number of mice alive and tumor-free at end of experiment	Mean age at death of nontumor mice (weeks)
			Number	Per cent	Mean age at appearance (weeks)		
N42	11	48	26	54	74 ± 2.8	6	76 ± 5.1
N44	8	49	6	12	81 ± 8.6	20	77 ± 3.6
N45	7	50	0	0		29	83 ± 2.8

* Number of mice alive when first tumor was observed: mice 43 weeks old.

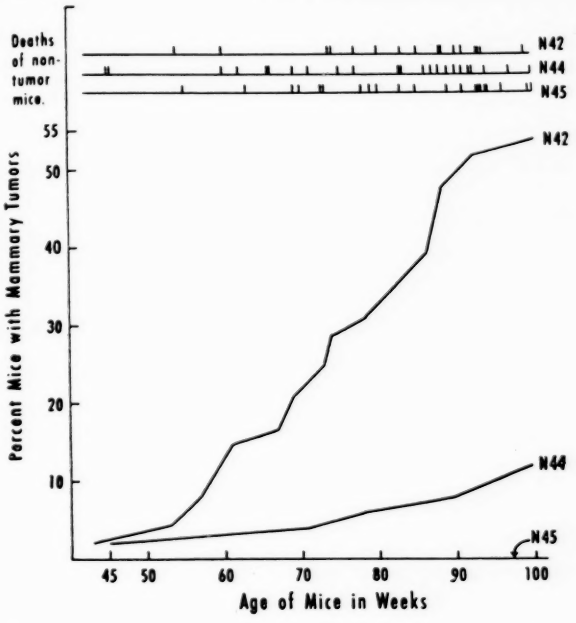

FIG. 1.—Cumulative curves illustrating dependence of formation of spontaneous mammary tumors on caloric intake. Chart at top of figure indicates age at death of individual mice without mammary tumors.

2.3 gm. (containing no added cornstarch). The composition of the diet, mean caloric intake, and mean weight of the mice are given in Table IIA.

This experiment was continued until the remaining mice were 134 to 138 weeks of age; only 4 mice were alive at this time, 3 of them in Group c23. The data regarding the number and percentage of tumors, average age at time of tumor appearance, and nontumor deaths are given in Table IIB. Since there were unequal numbers and a different time distribution of nontumor deaths in this experiment, the percentages of tumors were calculated not only on the basis of the effectual number of mice but also on the basis of an adjusted total, described by Bryan and Shimkin (1), that accounts for the deaths of nontumor animals during the period in which tumors appear.

The cumulative curves of tumor formation for each of the 5 groups are shown in Fig. 2A. The time at which nontumor deaths occurred is also given. The relationship of tumor formation to the degree of caloric restriction *per se,* at various times in the experiment, is shown in Fig. 2B. It can be seen that the curves vary, depending on the time that the comparisons are made. However, at the end of the experiment (approximately 2½ years), only the most restricted group reveals a significant inhibition of tumor incidence.

The results of Experiment 1 suggest that for the spontaneous mammary tumor of the mouse there is a proportionality between the degree of simple caloric restriction (carbohydrate only) and the degree of inhibition of tumor formation. In addition, Experiment 2 suggests that the proportionality is not linear, but that there may be a threshold caloric intake at which level the inhibition is more effective. That is, starting at the *ad libitum* level the decrease in the daily intake of nearly 3 calories (c33 to c25) resulted in no significant decrease in tumor formation, while the

TABLE IIA: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 2

Group	Diet constituents (gm. per mouse per day)				Mean daily caloric intake	Percentage composition of diets				Mean weight of mice in gm.					
	Fox chow meal	Skimmed milk powder	Corn-starch	Total		Protein	Fat	Carbo-hydrate	Ash	Age in weeks 23	35	45	65	83	General * level
c33	1.4	0.9	1.0	3.3	11.7	20	2	63	5	25	28	28	27	29	28
c29	1.4	0.9	0.6	2.9	10.3	23	2	60	5	25	25	26	26	26	26
c27	1.4	0.9	0.4	2.7	9.6	24	3	58	6	25	25	24	25	25	25
c25	1.4	0.9	0.2	2.5	8.9	26	3	55	6	25	23	23	23	24	23
c23	1.4	0.9		2.3	8.1	29	3	52	7	26	22	22	22	22	22

* Approximate mean weight from about 23 to 83 weeks of age.

TABLE IIB: EFFECT OF CALORIC INTAKE ON THE FORMATION OF SPONTANEOUS MAMMARY TUMORS IN DBA PAROUS FEMALE MICE

Group	Mean caloric intake	Number of mice		Mice developing mammary tumors †			Mean age at appearance of tumors (weeks)	Mean age at death of nontumor mice (weeks)
		Effectual total	Adjusted * total	Number	Per cent (effectual)	Per cent (adjusted) *		
c33	11.7	30	26	22	73	85	75 ± 3.1	73 ± 9.8
c29	10.3	30	24	17	57	71	84 ± 5.3	83 ± 8.2
c27	9.6	30	28	19	63	68	75 ± 4.0	103 ± 6.4
c25	8.9	25	21	17	68	81	89 ± 4.2	86 ± 8.8
c23	8.1	25	24	9	36	37	76 ± 6.1	115 ± 5.7

* See text.

† Experiment terminated when surviving mice were 134 to 138 weeks of age.

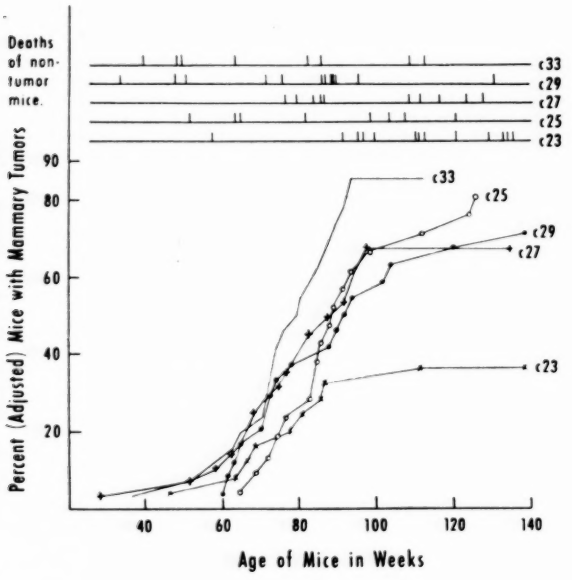

FIG. 2A.—Cumulative curves illustrating dependence of formation of spontaneous mammary tumors on caloric intake. Chart at top of figure indicates age at death of individual mice without mammary tumors.

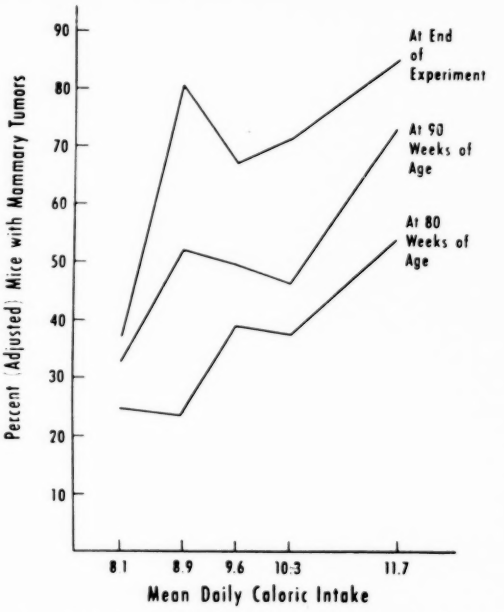

FIG. 2B.—Relationship between mean daily caloric intake and incidence of spontaneous mammary tumors.

TABLE IIIA: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 3

	Diet constituents (gm. per mouse per day)					Percentage composition of diets				Mean weight of mice in gm.						
										Weeks after 1st application of carcinogen						
Group	Fox chow meal	Skimmed milk powder	Corn-starch	Total	Mean daily caloric intake	Protein	Fat	Carbo-hydrate	Ash	−3	0	10	20	30	40	General * level
b40	1.4	0.9	1.7	4.0	14.3	17	2	68	4	22	27	33	36	33		34
b36	1.4	0.9	1.3	3.6	12.8	18	2	65	4	23	26	30	30	29		30
b33	1.4	0.9	1.0	3.3	11.7	20	2	63	5	23	26	29	28	27	27	28
b31	1.4	0.9	0.8	3.1	11.0	21	2	62	5	23	25	27	27	26	26	26
b29	1.4	0.9	0.6	2.9	10.3	23	2	60	5	22	24	26	25	25	25	25
b27	1.4	0.9	0.4	2.7	9.6	24	3	58	6	22	23	25	24	24	24	24
b25	1.4	0.9	0.2	2.5	8.9	26	3	55	6	23	23	23	22	22	23	22
b23	1.4	0.9		2.3	8.1	29	3	52	7	23	22	21	20	20	21	21

* Approximate mean weight during course of experiment after last application of carcinogen.

further decrease by only 0.8 calorie (c25 to c23) caused a substantial reduction in the percentage of tumors formed.

Experiment 3.—The mice comprising the 8 groups of this series were dba males, born between April 14 and April 27, 1942. Either 35 or 40 mice were placed in each group. After being on their respective diets for 3 weeks, when they were 10 to 12 weeks of age, the mice were given the first of 18 applications of a 0.3 per cent solution of 3,4-benzpyrene in acetone. The solution was applied in the interscapular area by means of a dropping pipette. Of the 18 semiweekly applications, the first 2 and last 4 consisted of double drops; the others were single drops.

The daily rations, which were comparable to those used in the previous experiment, contained 1.4 gm. fox chow meal and 0.9 gm. skimmed milk powder per mouse. Cornstarch was added to the several diets in decreasing amounts, graded from 1.7 gm. for the daily ration of the *ad libitum* group to none for the most restricted group. The composition of the diet, mean caloric intake, and mean weight of the mice are presented in Table IIIA.

The mean weight of the mice when transferred to the experimental diets ranged from 22.1 to 23.0 gm. As was to be expected, graded caloric intake produced graded weight levels, and these are given for various periods of the experiment.

The experiment proceeded smoothly; this is indicated by the fact that in most groups only 1 or 2 mice died of nontumor causes. Most of the tumors appeared as papillomas, and a high proportion of these became carcinomas. However, a few tumors in each group were first recognized as carcinomas, and in some groups 1 or 2 of the initial lesions were myxomas or sarcomas of the skin. In Table IIIB the data are pre-

sented both in terms of mice developing skin tumors of all kinds (papilloma, carcinoma, myxoma, or sarcoma) and of mice developing carcinomas only. The experiment was terminated 53 weeks after the first application of the carcinogen.

It can be seen that the final percentages of tumors in the 6 groups at the higher caloric levels are relatively comparable, while the 2 groups consuming the lower caloric intake show a significantly smaller incidence. Such a relationship may be expected with the relatively high dose of carcinogen employed in this experiment. The inhibitory effect of a restricted diet on the *incidence* of skin tumors can be masked by the use of a sufficiently high dose of carcinogen;[1] it is possible that such was the case with Groups b36, b33, b31, b29, and b27, since all reached or approached the 100 per cent level of tumor formation. If this is so, it is of interest that the effect of greater degrees of caloric restriction (b25 and b23) was still evident.

The relationship between caloric intake and the incidence of skin tumors at the end of the experiment is graphically presented in Fig. 3A. In addition, similar curves are given for two earlier periods; these latter curves afford an indication of results that may be expected with lower dosages of carcinogen. The corresponding curves for carcinomas follow the same pattern. Further evidence of a proportionality between the degree of caloric restriction and tumor formation, which apparently persists even at relatively high doses

[1] Unpublished data.

TABLE IIIB: EFFECT OF CALORIC INTAKE ON FORMATION OF BENZPYRENE SKIN TUMORS IN DBA MALE MICE

Group	Mean caloric intake	Number of mice (effectual total)	Per cent mice with skin tumors Weeks after 1st application of carcinogen 10	14	18	22	26	30	34	38	42	46	End of expt. 53	Time of appearance of skin tumors (weeks) Range	Mean	Mice with carcinomas Per cent	Time of appearance (weeks) Range	Mean	Mice tumor-free and alive at end of experiment (53rd week)
b40	14.3	40 *	8	38	63	75	80	88	93	95			95	8-36	18 ± 1.1	93	14-46	26 ± 1.4	0
b36	12.8	40	3	20	48	68	78	90	100				100	8-34	20 ± 1.1	98	16-48	29 ± 1.3	0
b33	11.7	40	0	18	35	43	53	75	80	85	90		90	12-40	23 ± 1.4	85	18-48	31 ± 1.4	1
b31	11.0	40	0	10	23	35	45	68	78	85	88	95	98	12-50	27 ± 1.6	80	14-53	33 ± 1.9	0
b29	10.3	40	3	13	23	43	55	68	70	80	88	90	90	10-46	25 ± 1.6	80	15-50	32 ± 1.8	2
b27	9.6	40	3	18	28	30	38	50	70	78	93	93	95	10-53	28 ± 1.8	90	14-52	35 ± 1.8	1
b25	8.9	35	0	3	9	11	14	17	23	31	49	60	66	12-53	36 ± 2.4	43	22-53	39 ± 3.0	10
b23	8.1	35 *	0	3	6	11	14	14	20	26	37	51	71	12-53	39 ± 2.4	54	16-52	40 ± 2.8	6

* Two mice without tumors were removed at 17th week for study of skin sections.

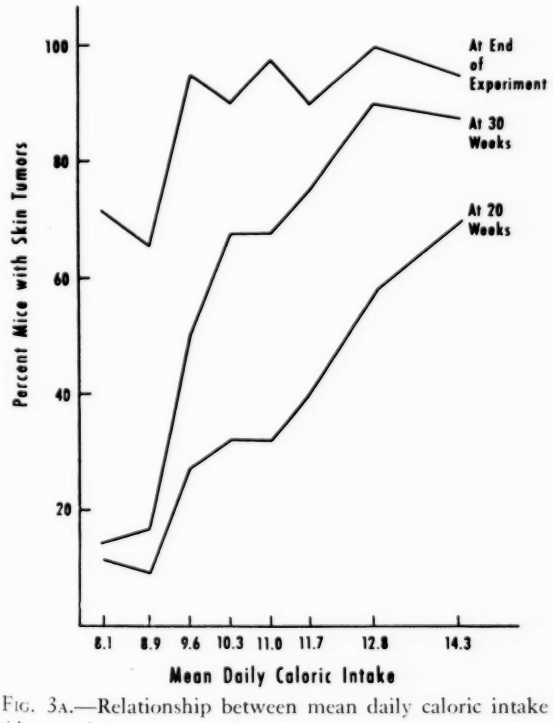

FIG. 3A.—Relationship between mean daily caloric intake and incidence of benzpyrene skin tumors.

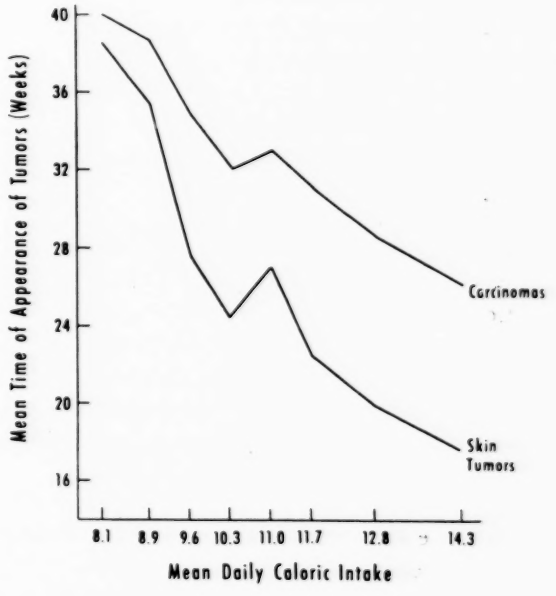

FIG. 3B.—Relationship between mean daily caloric intake and mean time of appearance of benzpyrene skin tumors and carcinomas.

of carcinogen, is given by the data on induction time (Table IIIB, Fig. 3B).

DISCUSSION

Caloric restriction *per se* in which the restricted group is limited to about 60 per cent of the intake of the *ad libitum* control group definitely inhibits the formation of tumors in the mouse. The question arises: What happens to tumor formation if mice are restricted to a caloric intake between the 100 and 60 per cent levels? The experiments reported in this paper suggest the possibility that as the caloric level is gradually reduced from the *ad libitum* level (100 per cent intake) there is little or no inhibition of tumor formation until the restricted diet approaches 65 to 70 per cent of the *ad libitum* intake; at these later levels there is a considerable inhibition of tumor formation.

However, some of the observations in Experiment 3 do not support this view. Two criteria of tumor formation, incidence and average time of appearance, were considered. It is to be noted that in Experiment 3, on induced skin tumors, there is a relatively gradual increase in the average induction time as the restriction is increased (Fig. 3B). Also, the profiles (Fig. 3A) of tumor formation at times before the experiment was terminated suggest that a gradation in percentage of tumors may be expected at lower doses of carcinogen than that employed.

It may be of importance to know whether the relationship of the inhibition of tumor formation to the degree of caloric restriction *per se* is a linear one, or if at a particular level of dietary restriction there is a sharper inhibition than occurs at other levels. The findings reported here, coupled with the results of experiments described in a following publication (7), suggest that the latter is more probable. Referring to caloric levels maintained throughout an experiment, it is likely that as the caloric content of the diet is reduced there is a gradual inhibition of tumor formation. Then, as dietary restriction reaches a critical level, a greater degree of inhibition occurs as further dietary restriction is imposed. Below this, further caloric restriction again results in a less striking decrease in tumor formation. A tentative curve of the relationship of graded caloric restriction *per se* to the formation of spontaneous mammary or induced skin tumors is given in Fig. 4. Although several experiments have been performed, it is our opinion that the data do not permit a more exact description of the relationship.

Modifying factors.—In any one experiment the curve expressing the relationship between tumor formation and the degree of caloric restriction *per se* is probably dependent on many factors. The type of tumor, and the time relationship between carcinogenic stimuli and the appearance of tumors, may play an important role. The carcinogenicity of the inciting agent and the dosage involved undoubtedly produce modifications of the curve. When the carcinogenic dose is high, it is likely that there will be a flattened upper limb near the 100 per cent tumor level. On the other hand, low dosages may produce a curve flattened near the 0 per cent level. At the present time the evidence tentatively suggests that the curve has a modified ∫ shape. Under certain experimental conditions this may be quite obvious, while under others it may be difficult to distinguish it from a linear relationship, particularly if only a few scattered levels of caloric restriction are studied.

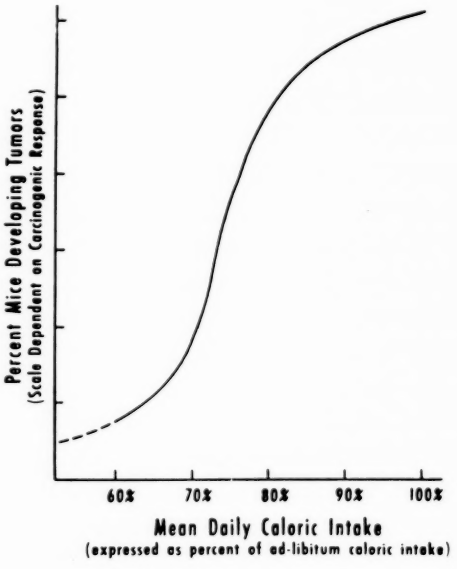

FIG. 4.—*Tentative composite curve* showing dependence of tumor incidence on degree of caloric restriction *per se* (restriction in carbohydrate only). Based on experiments in this and following paper (7).

Extent of caloric effect.—Caloric restriction of a small degree may reveal its inhibitory effect on tumor formation either through a decrease in the number of tumors or an increase in the average time of appearance, or both. When the caloric intake is greatly restricted (approximately 6 to 7 calories daily) and the carcinogenic stimulus is moderate, no tumors may appear. However, it is more important to stress the general effect of caloric restriction than to emphasize the sensational effect (no tumors at all) that may occur through a combination of proper experimental conditions and drastic caloric restriction.

Mechanism of inhibitory effect of caloric restriction.—That caloric restriction inhibits the formation of many types of tumors in the mouse is a fact, though its mechanism is still obscure. In previous publications (4, 5) we have discussed the possible mechanism of tumor inhibition caused by a calorie-restricted diet.

Since all tumors studied are affected in the same way, it was pointed out "that the intermediary factors causing this inhibiting effect are general in nature (possibly an effect on the whole endocrine-metabolic complex), present in all the tissues of the body, and effective at all sites investigated." It is probable that the inhibitory action is mediated through the consumption of a caloric intake lower than the actual requirements for "normal" growth and function. If the requirements are increased through some means (individual characteristics of the animals, exercise, drugs, etc.) a relatively "normal" caloric intake becomes inadequate. Under these conditions, even though food consumption be what is generally accepted as "normal," the animal's metabolic requirements are not being supplied, and an inhibition of tumor formation may result. Thus the inhibitory effect is probably dependent on the caloric intake relative to the animal's "needs," and not on the absolute intake.

Evidence has been presented (6) suggesting that the initial change preceding the development of a tumor occurs even with a restricted nutritive supply. However, energy and essentials over and above a basic level may be required before the initiated or biased cells can develop into a tumor. The results presented in this paper suggest the possibility that the basic level previously mentioned is associated with the dietary (caloric) range at which there is a relatively large change in tumor formation with comparatively small changes in caloric intake. Under the conditions of the present experiments this dietary range is approximately 2.1 to 2.4 gm. daily for females and 2.3 to 2.6 gm. daily for males.

The present experiments further support the suggestion that, in experimental cancer research, one must consider the nutritional aspects of an experiment as a possible modifying factor—the possibility that an experimental procedure may produce, secondarily, a restricted food intake or assimilation of sufficient degree to affect tumor formation.

SUMMARY

The effect of different levels of caloric intake on the formation of spontaneous mammary and induced skin tumors in the mouse was studied by grading the amount of carbohydrate in the diet (caloric restriction *per se*). Using as criteria both the incidence of tumors and the average time at which these appeared, it was found that inhibition of tumor formation is dependent on the degree of caloric restriction: The lower the caloric intake the greater the inhibition. In the experiment on induced skin tumors there is a progressive increase in the mean time of appearance (latent period) as the caloric level is decreased. It is probable that the curve expressing the relationship between tumor incidence and caloric intake has a modified ∫ shape; the ratio of the decrease in incidence to the decrease in caloric intake is greater in a particular caloric range.

REFERENCES

1. Bryan, W. R., and Shimkin, M. B. Quantitative Analysis of Dose-Response Data Obtained with Carcinogenic Hydrocarbons. J. Nat. Cancer Inst., **1**:807-833. 1941.
2. McCay, C. M., Ellis, G. H., Barnes, L. L., Smith, C. A. H., and Sperling, G. Chemical and Pathological Changes in Aging and after Retarded Growth. J. Nutrition, **18**: 15-25. 1939.
3. Saxton, J. A., Jr., Boon, M. C., and Furth, J. Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. Cancer Research, **4**:401-409. 1944.
4. Tannenbaum, A. The Initiation and Growth of Tumors. Introduction. I. Effects of Underfeeding. Am. J. Cancer, **38**:335-350. 1940.
5. Tannenbaum, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction per se. Cancer Research, **2**:460-467. 1942.
6. Tannenbaum, A. The Dependence of the Genesis of Induced Skin Tumors on the Caloric Intake during Different Stages of Carcinogenesis. Cancer Research, **4**:673-677. 1944.
7. Tannenbaum, A. The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction. Cancer Research, **5**: 616-625. 1945.
8. Visscher, M. B., Ball, Z. B., Barnes, R. H., and Sivertsen, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.
9. White, Florence R., White, J., Mider, G. B., Kelly, Margaret G., and Heston, W. E. Effect of Caloric Restriction on Mammary-Tumor Formation in Strain C3H Mice and on the Response of Strain dba to Painting with Methylcholanthrene. J. Nat. Cancer Inst., **5**:43-48. 1944.

The Dependence of Tumor Formation on the Composition of the Calorie-Restricted Diet as Well as on the Degree of Restriction

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The inhibitory effect of caloric restriction on the formation of tumors in the mouse has been shown (11) to be dependent on the actual degree of caloric restriction. Previously it had been demonstrated (8) that restriction of caloric intake to about 60 per cent of the *ad libitum* intake causes an inhibition in tumor formation regardless of whether only the carbohydrate component is restricted (so-called caloric restriction *per se*) or an aliquot of the *ad libitum* ration is fed (restriction of all components). The question arose whether these two types of restricted diets affect tumor formation to the same extent. Also, since it is known that a fat-enriched diet enhances the formation of spontaneous mammary and induced skin tumors (1, 9) it was of interest to determine the effect of high-fat but restricted diets on tumor formation.

The present experiments, in which spontaneous mammary and induced skin tumors of the mouse were utilized, were performed in order to gather more data regarding the effect on tumor formation of the *degree* of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the *composition* of the diet (at various caloric levels). Three types of restricted diets were used: (a) diets in which caloric restriction was achieved by decreasing the carbohydrate component only; (b) diets in which caloric restriction was achieved by reducing all components of the control ration (not necessarily in proportion to the caloric restriction); and (c) diets equivalent to the latter except that the fat content was increased by equicaloric substitution of fat for carbohydrate.

METHODS

The general methods, conditions, and criteria have been described (11). The special characteristics will be given under the separate investigations.

The constituents of the diets used in these experiments were Purina fox chow meal, skimmed milk powder, cornstarch,[1] and hydrogenated cottonseed oil.[2] The fox chow meal and skimmed milk powder contained the protein, vitamins, and minerals, and (as far as growth is concerned) adequate essential fatty acids. Consequently, variation or equivalence of these two materials in the diets is referred to as variation or equivalence of "essential components." The cornstarch and hydrogenated cottonseed oil utilized are practically free of vitamins, ash, and proteins; they were added to vary the caloric level and fat content of the diets. The compositions and caloric values of the diets were calculated from data supplied by the manufacturers of the foodstuffs.

It must be recognized that when caloric restriction *per se* is achieved by reducing the amount of cornstarch, there is concomitantly an increase in the percentage (not in the amount) of the essential components of the diet. Moreover, when cornstarch is replaced by an equicaloric amount of fat, the other constituents remaining constant, there is a decrease in the weight of the diet and a consequent increase in the proportion of the essential components. These latter points can be seen in the tables in which the diets are described.

EXPERIMENTAL

Experiment 1.—The 3 groups of this experiment were composed of either 50 or 54 dba male mice, all born between April 14 and May 23, 1941. They were placed on the respective experimental diets when they were 5 to 11 weeks old. Two weeks later they were given the first application of a 0.3 per cent solution of 3,4-benzpyrene in benzene. The solution was applied in the interscapular area by means of a dropping pipette.

The factors and conditions of the experiment were alike for all three groups, except for the differences in diet (Table IA) and the number of semi-weekly applications of the carcinogen. The mice of the restricted groups, x5 and x25, received 25 applications (in 12 weeks), whereas those of the *ad libitum* group, x12 were given only 17 (in 8 weeks). There were special reasons for this procedure: the x12 group was also being used as a control (for other experi-

[1] Anheuser-Busch cornstarch 712-87.

[2] Kremit (partially hydrogenated cottonseed oil) generously furnished by Armour & Co.

mental groups carried on at the same time) in which only a moderate tumor response was desired. Seventeen applications of the carcinogen solution would have produced only a few tumors in the restricted groups; therefore, these two groups were given 25 applications in order that the tumor response would be in a range where differences between the restricted groups, if any, would be more readily recognized.

The composition of the diet, mean caloric intake, and mean weight of the mice are given in Table Ia.

TABLE Ia: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 1

	Diet constituents (gm. per mouse per day)					Percentage composition of diets				Mean weight of mice in gm.								
										Weeks after 1st application of carcinogen								
Group	Fox chow meal	Skimmed milk powder	Corn-starch	Total	Mean daily caloric intake	Protein	Fat	Carbo-hydrate	Ash	−2	0	8	12	20	30	40	50	General † level
x12	1.4	0.9	1.9	4.2	13-14 *	16	2	69	4	24	28	34	37	38	39	38		38
x5	1.4	0.9		2.3	8.1	29	3	52	7	25	23	22	21	22	22	21	21	21
x25	0.8	0.5	1.0	2.3	8.2	16	2	68	4	24	22	24	22	22	22	21	22	22

* Calculated from food consumption, which varied during course of experiment.

† Approximate mean weight during course of experiment following last application of carcinogen.

TABLE Ib: EFFECT OF COMPOSITION OF A CALORIE-RESTRICTED DIET ON FORMATION OF BENZPYRENE SKIN TUMORS IN DBA MALE MICE

			Mice developing skin tumors				
Group	Number of mice (effectual total) *	Number of applications of carcinogen	Number	Per cent	Mean time of appearance (weeks)	Per cent mice developing carcinomas	*Number of mice tumor-free and alive at end of experiment (54th week)
x12	54	17	35	65	34 ± 2.6	46	17
x5	48	25	12	25	38 ± 6.1	13	33
x25	49	25	19	39	34 ± 7.1	27	23

* Number of mice alive at 13th week of experiment.

It is to be noted that, in comparison with x12, x5 was restricted only in carbohydrate. On the other hand, group x25 was fed a ration prepared by restricting all components (essentials as well as calories) of the x12 diet. The two restricted diets were approximately equicaloric.

The control mice grew normally, while those of the two restricted groups maintained a fairly constant weight; the mean weights of the latter groups were approximately the same. The experiment ran for 54 weeks. Table Ib contains the data on tumor incidence and average time of appearance.

At the termination of the experiment the carbohydrate-restricted group (x5) had 25 per cent skin tumors in comparison with 39 per cent for the all-component-restricted group (x25). These compare with 65 per cent for the "control" group (x12). While a direct comparison can be made only between x5 and x25, the x12 group may be considered a qualitative control, particularly since more tumors were formed here than in the restricted groups in spite of fewer applications of the carcinogen. With regard to the formation of carcinomas the percentages for x5, x25, and x12 are 13, 27, and 46, respectively. The curves of tumor formation are given in Fig. 1.

Although caloric restriction inhibits tumor formation, the method by which the restriction is achieved apparently modifies the degree of the effect. It appears that the mice receiving the diet restricted in carbohydrate only developed fewer tumors, and at a later mean time of appearance, than did the mice receiving the diet restricted in all components.

Experiment 2.—Four groups of 50 virgin dba female

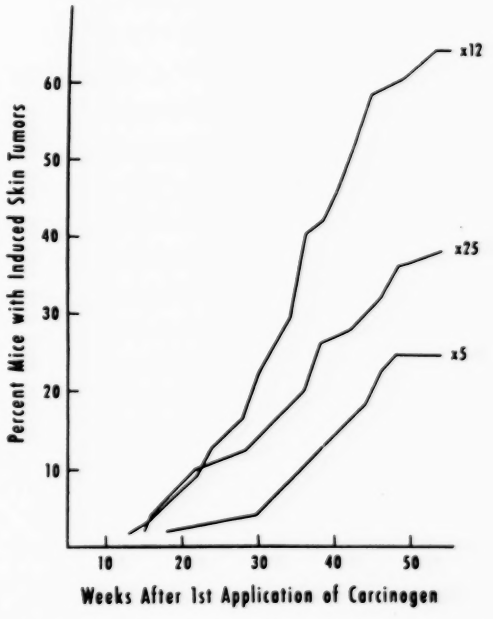

FIG. 1.—Cumulative curves of formation of induced skin tumors, illustrating effect of composition of diet on inhibition caused by caloric restriction. x12, *ad libitum* control; x5, restricted in carbohydrate only; x25, restricted in all components.

mice, all born between April 14 and 23, 1941, were utilized in this experiment. The groups were constructed by litter-mate distribution. After weaning, the mice were fed Purina fox chow checkers until they were 9 to 10 weeks of age, when the experimental diets were instituted; these were respectively, *ad libi-*

TABLE IIA: DIET, MEAN CALORIC INTAKE, AND MEAN WEIGHT OF MICE OF EXPERIMENT 2

Group	Diet constituents (gm. per mouse per day)					Mean daily caloric intake	Percentage composition of diets				Mean weight of mice in gm.						
	Fox meal chow	Skimmed milk powder	Corn-starch	Kremit	Total		Protein	Fat	Carbo-hydrate	Ash	Age in weeks 10	30	50	70	90	110	General † level
T2	1.2	0.8	1.4		3.4	11-12 *	17	2	67	4	22	32	34	35	32	31	33
T5	1.2	0.8	0.7		2.7	9.6	21	2	62	5	21	22	25	24	23	24	24
T25	0.7	0.5	1.5		2.7	9.7	13	2	73	3	22	21	24	24	22	23	24
T15	0.7	0.5	0.5	0.32	2.0	8.9	17	18	53	4	21	22	25	25	22	24	24

* Calculated from food consumption, which varied during course of experiment.
† Approximate mean weight between 50 and 110 weeks of age.

TABLE IIB: FORMATION OF SPONTANEOUS MAMMARY TUMORS IN DBA VIRGIN FEMALE MICE: MODIFICATION OF INHIBITORY EFFECT OF CALORIC RESTRICTION BY COMPOSITION OF RESTRICTED DIET

Group	Diet characteristics			Number of mice (effectual total) †	Mice developing mammary tumors by end of experiment (136 weeks)			Mean age at death of non-tumor mice (weeks)
	Mean daily caloric intake	Relative * amount of "essentials"	Per cent fat		Number	Per cent	Mean age at appearance (weeks)	
T2	11.5	1.0	2	47	35	74	89 ± 3.1	74 ± 9.7
T5	9.6	1.0	2	49	23	47	99 ± 3.6	102 ± 5.2
T25	9.7	0.6	2	49	32	65	86 ± 3.3	89 ± 5.5
T15	8.9	0.6	18	47	41	87	80 ± 3.2	71 ± 11.5

* Relative amount of protein, vitamins, and minerals: T2 = 1.
† Number of mice alive in group when first tumor appeared: mice 45 weeks old.

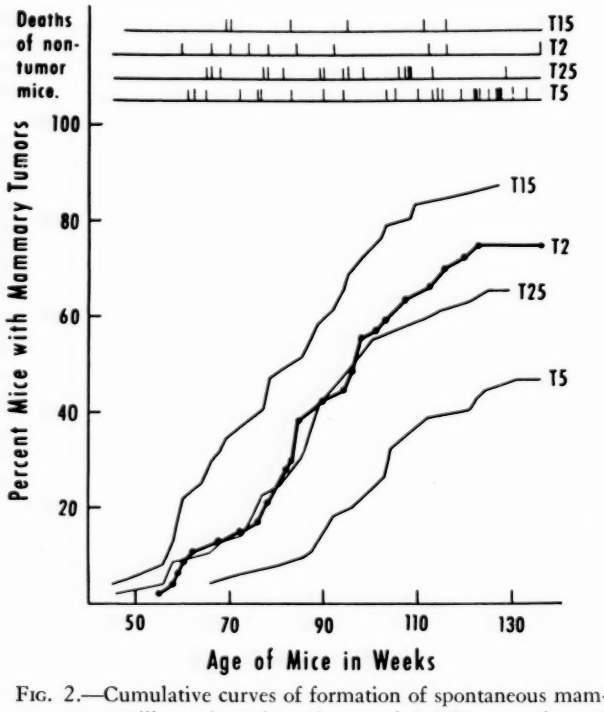

FIG. 2.—Cumulative curves of formation of spontaneous mammary tumors illustrating dependence of inhibitory effect of caloric restriction on composition of restricted diet. T2, *ad libitum* control; T5, restricted in carbohydrate only; T25, restricted in all components (low fat); T15 similar to T25 but with high fat content.

tum (T2), calorie (carbohydrate) restricted (T5), all components restricted (T25), and the latter with fat substituted for some of the carbohydrate (T15). The 3 restricted diets were approximately equicaloric. The composition of the diet, mean caloric intake, and mean weight of the mice are shown in Table IIA.

The experiment was terminated at a time when only 3 mice were alive (136 weeks of age). The experiment ran smoothly and the mice were in good condition throughout. The data with regard to mammary tumor incidence and the time of appearance of these tumors are shown in Table IIB and Fig. 2.

The following results, in comparison with tumor production in the *ad libitum* control (T2), were obtained in the 3 restricted groups: The high-fat group (T15) developed more tumors and, on the average, at a significantly earlier time; the group restricted in essential components (T25) developed fewer tumors, but at about the same time; the group restricted in carbohydrate only (T5) developed significantly fewer tumors, and at a later time. The actual percentages of mammary tumors, on an effectual total basis, in the T2, T15, T25, and T5 groups were 75, 87, 66, and 47 respectively.

This experiment clearly indicates that although caloric restriction may inhibit tumor formation, the

components of the restricted diet are of great importance, for they may be of such a nature (*i.e.,* high-fat) that under proper conditions tumor production is enhanced over and above the control level. It is also obvious that the augmenting effect of the high-fat diet is not due to a caloric effect (since the 3 restricted groups were approximately equicaloric) but to some unexplained essential action of fat.

In both Experiments 1 and 2, diets restricted in carbohydrate only (caloric restriction *per se*) inhibited tumor formation to a greater extent than did an equicaloric diet restricted in all components. In Experiment 1, performed with induced skin tumors, and with diets restricted to approximately 60 per cent of the *ad libitum* intake, the differences were not statistically significant. On the other hand, in Experiment 2, performed with spontaneous mammary tumors and diets restricted to 80 per cent of the *ad libitum* intake, the differences were statistically significant. The agreement in results between the experiments supports the viewpoint that the nature of the restricted diet modifies the inhibitory effect on the formation of these two types of tumors in the mouse.

Graded caloric restriction using diets of different composition.—In Experiments 1 and 2 it was concluded that the composition of the restricted diet at a particular caloric level is a modifying factor for both the number of tumors and the average time at which they appear. In Experiments 3 and 4, which follow, the 3 types of restricted diets were studied at more than one caloric level.

The diets stemmed from a low-fat basic diet, 40, composed of Purina fox chow meal, skimmed milk powder, and cornstarch; this diet was sufficient in both essentials and calories to support good growth and health in the C3H male mice used in Experiment 3.

The types of diets were: (a) those constructed from basic diet 40 by restriction of cornstarch (calories) only; (b) those constructed from diet 40 but by restricting essentials (fox chow meal and skimmed milk powder) as well as cornstarch; (c) those comparable to the latter except that fat was substituted equicalorically for some of the cornstarch. The series may be briefly indicated as: (a) restricted in calories (carbohydrate) only; (b) restricted in calories and essentials (low in fat); (c) restricted in calories and essentials, but high in fat.

The diet and group numbers indicate the type of diet fed. Group numbers 40 and below received the carbohydrate-restricted diets; those of the 200 series were given the diets restricted in essentials as well as carbohydrates; the 100 series were fed the high-fat diets. The last 2 figures of the group or diet number indicate the caloric level of the diet. For example, diets 40 and 140 were equicaloric, as were diets 28, 128, and 228.

The diets utilized in Experiments 3 and 4 are presented in Table IIIA.

TABLE IIIA: COMPOSITION OF DIETS USED IN EXPERIMENTS 3 AND 4

Diet number	Fed to groups: In experiment 3 i-series	Fed to groups: In experiment 4 h-series	Constituents (grams per mouse per day): Fox chow meal	Skimmed milk powder	Cornstarch	Kremit	Total	Percentage composition of diet: Protein	Fat	Carbohydrate	Ash	Calories per mouse per day
40	i40		1.4	0.8	1.8		4.0	16	2	69	4	14.3
33	i33	h33a & b	1.4	0.8	1.1		3.3	19	2	65	4	11.7
28	i28	h28	1.4	0.8	0.6		2.8	22	2	60	5	10.0
26		h26	1.4	0.8	0.4		2.6	24	3	58	6	9.2
24	i24	h24	1.4	0.8	0.2		2.4	26	3	55	6	8.5
22		h22	1.4	0.8			2.2	28	3	52	7	7.8
228	i228	h228	1.2	0.7	0.9		2.8	19	2	64	5	10.0
226		h226	1.1	0.6	0.9		2.6	18	2	65	4	9.3
224	i224	h224	1.0	0.6	0.8		2.4	19	2	65	4	8.5
222		h222	1.0	0.5	0.7		2.2	19	2	64	5	7.8
140	i140		1.4	0.8	0.55	0.51	3.3	19	18	50	5	14.4
133		h133	1.4	0.8	0.10	0.42	2.7	23	18	45	5	11.9
128	i128	h128	1.2	0.7	0.05	0.35	2.3	23	18	45	5	10.1
126		h126	1.1	0.6	0.05	0.33	2.1	23	18	45	5	9.2
124	i124	h124	1.0	0.6	0.05	0.30	2.0	23	18	45	5	8.5
122		h122	1.0	0.5		0.28	1.8	23	18	44	6	7.8

Experiment 3.—This experiment was designed to test the effect of both graded caloric restriction and the composition of restricted diets on the formation of induced skin tumors. The nine groups were each composed of 50 C3H male mice (born between April 19 and July 17, 1943), and were equivalent as to age and weight of the mice. The diets were instituted when the mice were 5½ to 16 weeks of age. Two

TABLE IIIB: MEAN WEIGHT IN GRAMS OF MICE OF EXPERIMENT 3

Group	Weeks after 1st application of carcinogen							General * level
	−2 †	0	9	19	29	39	49	
i40	24	27	31	34	34	34	35	34
i33	24	25	26	26	26	28	29	27
i28	24	22	23	23	25	26	24	25
i24	24	20	19	19	21	22	20	20
i228	24	22	23	23	23	24	23	23
i224	24	20	20	20	20	20	20	20
i140	24	28	31	34	34	33	37	34
i128	24	23	23	24	23	24	23	24
i124	24	21	20	19	20	20	21	20

* Approximate mean weight during course of experiment following last application of carcinogen.

† Mean weight when experimental diets were instituted; 2 weeks before first application of carcinogen.

In each of the 3 series, tumor incidence is progressively decreased with progressive caloric restriction of the diet. There is also a tendency for the tumors to appear later, on the average, as restriction is increased. In addition, at a particular level of caloric restriction, the high-fat diet (i140, 128, or 124) permits the formation of a higher percentage of tumors than its low-fat counterpart (i40, 228, or 224). While the difference at each level is not statistically significant, the augmentation is consistent and in agreement with the results of other experiments. The lack of difference in this experiment between the effect of diets restricted in carbohydrate only (i24 and 28) and the corresponding equicaloric diets restricted in all components (i224 and 228) will be discussed later. These points are more readily seen in Fig. 3, in which the incidence of tumors as a function of both caloric intake and composition of diet is graphically presented.

TABLE IIIC: FORMATION OF METHYLCHOLANTHRENE SKIN TUMORS AS AFFECTED BY COMPOSITION OF DIET AND CALORIC INTAKE

Group	Diet characteristics			Number † of mice (effectual total)	Mice developing skin tumors by end of experiment (55 weeks)				Per cent mice with carcinomas	Mice tumor-free and alive at end of experiment
	Mean caloric intake	Relative * amount of "essentials"	Per cent fat		Number	Per-cent	Time of appearance, (weeks) ‡ Range	Time of appearance, (weeks) ‡ Mean		
i40	14.3	1	2	50	43	86	5-55	32 ± 1.9	60	4
i33	11.7	1	2	50	29	59	11-53	37 ± 2.4	38	17
i28	10.0	1	2	50	17	34	21-55	41 ± 2.4	22	24
i24	8.5	1	3	46	7	15	23-55	38 ± 4.5	14	24
i228	10.0	0.86	2	49	18	37	19-53	39 ± 2.1	22	23
i224	8.5	0.73	2	45	9	20	13-53	41 ± 4.1	16	26
i140	14.4	1	18	50	46	92	11-55	29 ± 2.1	78	3
i128	10.1	0.86	18	49	27	55	17-55	40 ± 2.1	41	19
i124	8.5	0.73	18	48	14	29	17-55	41 ± 2.8	23	25

* Relative amount of protein, vitamins, and minerals: i40 = 1.

† Number of mice alive at fifth week of experiment.

‡ Weeks after first application of carcinogen.

weeks later the first of 10 weekly applications of carcinogen was given: one drop of a 0.3 per cent solution of methylcholanthrene in acetone applied with a dropping pipette to the interscapular area.

The experimental diets have been discussed, and are listed in Table IIIA. The control diets, low-fat (i40) and high-fat (i140), contained equal amounts of essential components, and were approximately equicaloric. During the major part of the experiment most of the mice fed these 2 diets ate all the daily ration. The restricted mice consumed all their daily ration throughout the experiment.

At the beginning of the experiment the mean weights of the 9 groups ranged from 23.7 to 24.4 gm. The mean weight of the mice at various times of the experiment is shown in Table IIIB.

The experiment was terminated 55 weeks after the first application of the carcinogen. The data regarding the incidence and mean time of appearance of skin tumors are given in Table IIIc.

An incidental finding is the data on hepatomas, which develop spontaneously in the males of strain C3H. The first one was seen in a 45 week old mouse of the i140 group. Of 34 mice of i140 group (high-fat control) sacrificed between 45 and 73 weeks of age, 12 (35 per cent) were found to have hepatomas. In contrast, hepatomas were found in only 3 of 32 mice (9 per cent) in the i40 group (low-fat control) sacrificed during the same interval; the first hepatoma observed in this group was in a mouse 65 weeks old. In the 7 restricted groups, 158 mice were sacrificed between 62 and 73 weeks of age; only 2 had hepatomas. Apparently, as in the case of the induced skin tumor and spontaneous mammary tumors, the formation of a spontaneous hepatoma in C3H male mice is inhibited by caloric restriction, and augmented by a high-fat diet.

Experiment 4.—This experiment was designed as a companion investigation to Experiment 3, but in this

instance spontaneous mammary tumors were studied. The 15 groups were each composed of 30 virgin C3H females (born between April 19 and July 17, 1943) and were equivalent as to age and weight. The experimental diets were instituted when the mice were from 5 to 15 weeks of age. As in Experiment 3, the 3 diet types were studied at several caloric levels (Table IIIA). In this experiment diet 33 served as control, since it is at the *ad libitum* level for C3H female mice; throughout the major portion of the experiment most of the mice being fed this diet consumed all the daily ration. The mice receiving the

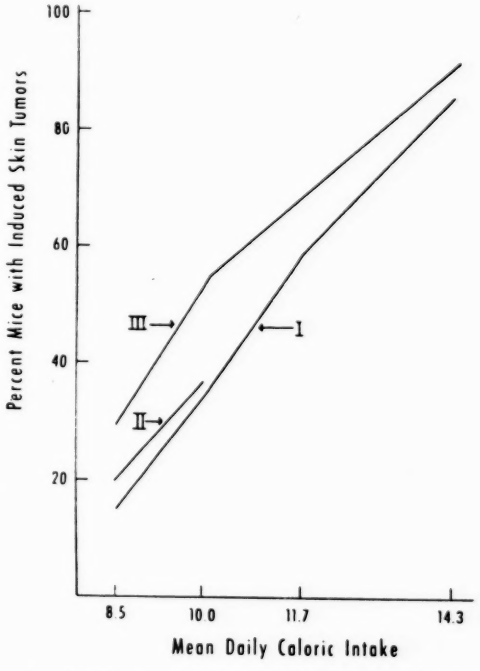

FIG. 3.—Incidence of methylcholanthrene skin tumors: dependence on composition of diet and caloric intake. Curve I—diets varying in carbohydrate content (caloric restriction *per se*); Curve II—diets varying in all components (with low-fat content); Curve III—diets varying in all components but with high-fat content.

restricted diets consumed all their daily ration throughout the experiment.

Because of inherent difficulties in constructing 15 uniform groups, two separate groups (h33a and h33b) were used as controls and were fed diet 33. This was done in order to obtain an impression, at least, of the variation to be expected between any 2 groups when such variation arises because of the manner of selecting mice for the individual groups of the experiment and other random factors.

Again, as in the previous experiments, graded caloric restriction produced graded weight levels. The mean weight of the mice of the various groups is shown in Table IVA.

Laboratory conditions prompted the termination of the experiment when the youngest mice were 64 weeks of age. The percentages of tumors formed by the 64th week, on an effectual total basis, are given in Table IVB. Although further extension of the experiment would have increased these percentages in some groups, previous experience (including the results of Experiment 2) suggests that the main differential results would be maintained.

The difference in tumor formation between the two control groups, h33a and h33b, is well within the expected limits, suggesting that with respect to the potentiality of tumor formation the 15 groups were relatively uniform at the beginning of the experiment.

As in Experiment 3, it can be noted that the greater the degree of caloric restriction in each of the 3 series,

TABLE IVA: MEAN WEIGHT IN GRAMS OF MICE OF EXPERIMENT 4

Group	Age in weeks						General * level
	10	20	30	40	50	60	
h33a	21	24	27	26	30	31	28
h33b	20	25	27	27	30	32	28
h28	20	21	22	23	24	24	23
h26	21	20	21	22	24	25	22
h24	21	20	19	20	20	21	20
h22	21	18	17	18	18	18	18
h228	21	21	23	23	25	25	24
h226	21	20	21	21	23	24	23
h224	21	20	19	21	22	22	21
h222	21	18	17	18	17	17	17
h133	21	25	27	26	27		27
h128	21	22	23	23	25	25	24
h126	21	21	23	23	25	25	24
h124	20	20	19	20	21	22	20
h122	21	18	18	19	20	21	19

* Approximate mean weight between 20 and 60 weeks of age.

the greater the inhibition of tumor formation. Again, at the levels of caloric intake studied, the high-fat diets (100 series) caused the formation of more tumors than did the corresponding equicaloric low-fat diets (200 series).

The questionable existence of a difference in tumor formation between groups restricted only in carbohydrate and the corresponding groups restricted in all components will be taken up in the discussion.

In Fig. 4 the incidence of mammary tumors as a function of both caloric intake and composition (type of restriction) is graphically represented.

DISCUSSION

The results of Experiments 3 and 4 confirm the finding (11) that the inhibitory effect of caloric restriction *per se* (carbohydrate only) on the formation of spontaneous mammary and induced skin tumors of the mouse is dependent on the actual degree of restriction.

TABLE IVB: FORMATION OF SPONTANEOUS MAMMARY TUMORS (C3H VIRGIN FEMALE MICE) AS AFFECTED BY COMPOSITION OF DIET AND CALORIC INTAKE

Group	Diet characteristics: Mean caloric intake	Diet characteristics: Relative * amount of "essentials"	Diet characteristics: Per cent fat	Number † of mice (effectual total)	Mice developing mammary tumors by 64 weeks of age: Number	Mice developing mammary tumors by 64 weeks of age: Per cent	Age at appearance (weeks): Range	Age at appearance (weeks): Mean	Mice tumor-free and alive at 64 weeks of age
h33a	11.7	1	2	30	16	53	35-59	48 ± 1.5	14
h33b				30	19	63	35-64	45 ± 1.6	9
h28	10.0	1	2	30	8	27	41-63	52 ± 2.6	18
h26	9.2	1	2	30	2	7	45-47	46 ‡	25
h24	8.5	1	2	30	0	0			19
h22	7.8	1	2	29	0	0			22
h228	10.0	0.86	2	30	12	40	46-61	54 ± 1.4	18
h226	9.3	0.77	2	28	4	14	29-63	50 ‡	20
h224	8.5	0.73	2	29	0	0			24
h222	7.8	0.68	2	28	0	0			19
h133	11.9	1	18	30	27	90	22-54	42 ± 1.6	2
h128	10.1	0.86	18	30	20	67	27-64	50 ± 2.3	10
h126	9.2	0.77	18	30	18	60	33-64	50 ± 2.5	10
h124	8.5	0.73	18	29	5	17	41-63	53 ‡	18
h122	7.8	0.68	18	28	1	4	37		24

* Relative amounts of protein, vitamins, and minerals: h33 = 1.
† Number of mice alive in group when first tumor appeared in experiment: mice 22 weeks old.
‡ Little significance can be attached to these means because of the small number of tumors.

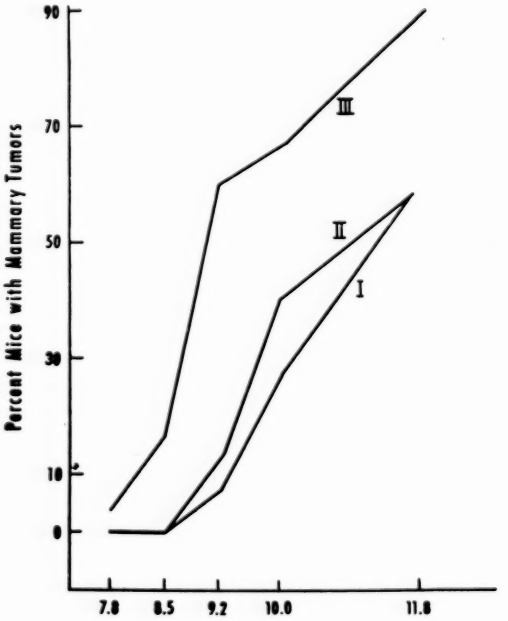

FIG. 4.—Incidence of spontaneous mammary tumors in C3H virgin female mice (at 64 weeks of age): dependence on composition of diet and caloric intake. Curve I—diets varying in carbohydrate content (caloric restriction *per se*); Curve II—diets varying in all components (with low-fat content); Curve III—diets varying in all components but with high-fat content.

This relationship obtains whether the calorie-restricted rations are: (a) restricted only in the amount of carbohydrate (caloric restriction *per se*); (b) restricted in essentials (protein, vitamins, and minerals) as well as in carbohydrate (low-fat content); or (c) restricted in essentials and carbohydrate, but with high fat content. In addition, the average time of appearance of the tumors is also dependent, in general, on the degree of restriction: The greater the caloric restriction the later the tumors appear.

In Experiment 4 the curves (Fig. 4) showing the relationship between tumor formation and caloric intake for the 3 different types of diets appear to have a modified ∫ shape. This is in general agreement with the previous conclusions regarding caloric restriction *per se* (11). On the other hand, the comparable curves of Experiment 3 (Fig. 3) appear to be linear, but this is probably due in part to the fact that fewer levels of caloric intake were studied.

Increasing the fat content of an *ad libitum* ration enhances the production of spontaneous mammary and induced skin tumors of the mouse. In this communication (Experiments 2, 3, and 4) it is further shown that there continues to exist an augmenting effect of a high-fat diet even at restricted levels of caloric intake.

Lavik and Baumann (1) investigated the differential effects of low-fat and high-fat diets at 2 levels of caloric intake, normal and restricted, on the production of skin tumors by methylcholanthrene. The incidence at each level of caloric intake was somewhat higher on the high-fat diet than on the corresponding low-fat diet. They interpreted this increase as possibly due to local contact of the fatty rations with the skin. Furthermore, they suggested that "much of the systemic or cocarcinogenic activity of dietary fat, if not all, is exerted through the medium of a voluntarily increased

intake of calories on diets high in fat." Our experiments do not support this view.

In earlier publications (9, 10) we have stated that the ingestion of a high-fat diet augments the formation of certain types of tumors mainly through a cocarcinogenic action on the developing tumor cell. Mice fed high-fat diets consumed more calories, possibly because of the greater compactness of the high-fat diet, and the augmentation in tumor formation may have been partly dependent on the increase in caloric intake. However, in the present experiments, such situations were obviated by the use of equicaloric diets which were fed at restricted levels where the mice consumed all food given them. The equicaloric consumption of the diets is evidenced by the rough equality in mean weight of corresponding groups. In every case where equicaloric diets were fed, the animals receiving the high-fat ration developed a higher incidence of tumors. Moreover, in the case of spontaneous mammary tumors, where mechanical contact with the fatty ration can not logically be assumed to be a factor, the augmenting effect of increased dietary fat is most pronounced; so great, in fact, that the groups in which the mice were fed diets approximately 85 per cent of the *ad libitum* caloric intake, but with a high-fat content, developed as many tumors as, or more than, the control groups on the *ad libitum* low-fat diet (T15 and T2; h128 and h33a or b).

Thus the evidence confirms our previous concept, that the enhancing effect of a high-fat diet on the formation of spontaneous mammary and chemically induced skin tumors is due mainly to some direct property of fat. What is important is that there exists an action of fat independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

In contrast with the definite increase in tumor formation produced by increasing the dietary fat at various caloric levels, there is only a questionable difference between the inhibitory effect of diets prepared by limiting essential components (as well as calories) and that produced by diets limited only in the carbohydrate component (caloric restriction *per se*). In Experiments 1, 2, and 4 it appears that caloric restriction *per se* (restriction in carbohydrate only) is more effective in inhibiting tumor formation than is the restriction of essentials as well as calories. Even though Experiment 3 does not support this viewpoint and, in general, the differences are not of great magnitude, it is probable that a difference does exist. This is seen more definitely in the experiments on spontaneous mammary tumors than in those utilizing induced skin tumors. In all our studies on the consequence of caloric restriction or high-fat diets on the formation of tumors we have observed that the effects were of greater magnitude with spontaneous mammary tumors than with induced skin tumors.

There are probably many modifying factors in experiments designed to test whether diets restricted in carbohydrate only or in essentials as well as calories have differential effects on tumor formation. Possible factors are: the type of tumor studied; the carcinogenicity of the inciting agent, and dosage; the composition of the control *ad libitum* diet; and the extent of the differences in essential components. In Experiments 1 and 2, for example, where larger differences in tumor formation were observed, the diets restricted in essential components contained 40 per cent less essentials than did the carbohydrate-restricted diets; on the other hand, in Experiments 3 and 4 the differences in essential components were smaller: 14 per cent less at the 10 calorie level, and 27 per cent less at the 8.5 calorie level.

Thus the *composition* of the restricted diet as well as the *degree* of restriction is of importance in determining the extent of inhibition of tumor formation produced through caloric restriction. A restricted diet with a high-fat content definitely results in the least inhibition of tumor formation in comparison with either (a) an equicaloric diet that differs only in that it is low in fat, or (b) an equicaloric diet containing more protein, vitamins, and minerals than (a). Until more evidence is presented, one may accept the view that between the latter 2 types of restricted diets, there is a difference in the effects, often very slight.

The results reported previously (11) combined with those recorded in this communication suggest the tentative curves given in Fig. 5, indicating the relationship of tumor formation to both the *degree* of restriction and the *composition* of the restricted diet.

These curves have been drawn for spontaneous mammary and chemically induced skin tumors of the mouse. They may be valid for other types of tumors also, but certainly not for all. For example, it is known (9) that a high-fat diet has little or no effect on the formation of spontaneous lung tumors of the mouse. Therefore the 3 curves (Fig. 5) for that particular type of tumor may, in reality, be reduced to only 2 separate curves or even one. It has been suggested also (9) that the induction of sarcomas by hydrocarbons may actually be inhibited by a high-fat diet; in this case the 3 curves may have entirely different relationships.

It is realized that both the experiments and interpretations regarding the effects of the degree of caloric restriction and the composition of the restricted diet refer to the effects produced by the *ingestion* of the various diets. The consequent differences in absorption, interconversion of fats and carbohydrate, sparing mech-

anisms,[3] and intermediary metabolism in general, are not sufficiently defined at present to afford an explanation for the observed effects on tumor formation.

At this time it seems advisable to summarize some of the evidence regarding a possible role of nutrition in the prevention of human cancer. Included are: (a) the inhibiting effect of caloric restriction on the formation of induced skin tumors (1, 5, 8), induced sarcoma (5, 8), spontaneous mammary tumors (5, 8, 12, 13), spontaneous lung tumors (5), and induced and spontaneous leukemia (4, 13) of the mouse, and spontaneous tumors of the rat (3); (b) the fact brought out in this communication, that some inhibition of the formation of induced skin and spontaneous mammary tumors is obtained even with reasonable degrees

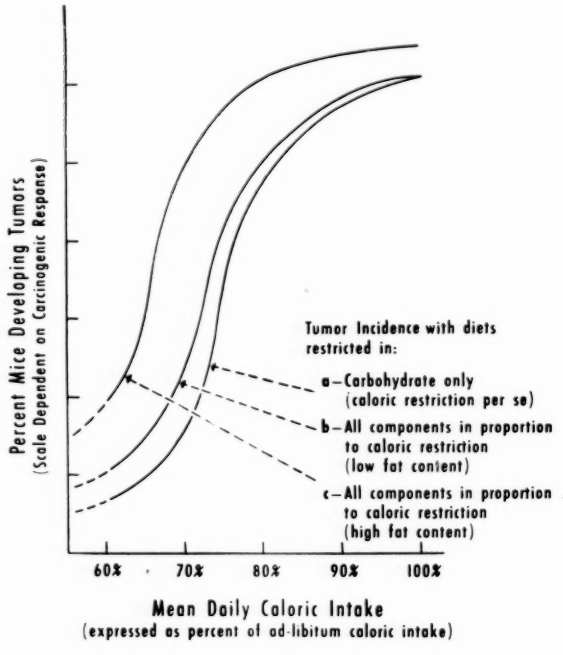
Percent Mice Developing Tumors
(Scale Dependent on Carcinogenic Response)
Tumor Incidence with diets restricted in:
a—Carbohydrate only (caloric restriction per se)
b—All components in proportion to caloric restriction (low fat content)
c—All components in proportion to caloric restriction (high fat content)
60%
70%
80%
90%
100%
Mean Daily Caloric Intake
(expressed as percent of ad-libitum caloric intake)

FIG. 5.—*Tentative composite curves* showing relationship of tumor incidence (spontaneous mammary and chemically induced skin tumors) to both level of caloric intake and composition of diet. Based on experiments in this communication and those of preceding paper (11).

of caloric restriction; (c) the inhibiting effect of a low-fat diet on the formation of induced skin tumors (1, 9), spontaneous mammary tumors (9), and spontaneous hepatoma (this paper); (d) the existence of a direct relation between the frequency of mammary carcinoma of the mouse and the average weight, the latter being greater in tumor-bearing mice (2); and (e) the data obtained from a study (6) of insurance statistics, indicating that overweight persons have a greater tendency to develop cancer.

[3] Phenomena like the action of carbohydrate in reducing the need for protein.

These observations support our previous conclusions (6, 7, 8, 10) that the avoidance of overweight through calorie-restricted and low-fat diets may aid in preventing many types of cancer in man, or at least delay the onset.

SUMMARY

The present experiments on spontaneous mammary and induced skin tumors in mice were performed to gather more data regarding the effect on tumor formation of the *degree* of caloric restriction, and at the same time to ascertain whether or not there is a differential effect due to the *composition* of the diet (at various caloric levels).

Further evidence was obtained that caloric restriction inhibits the formation of tumors, and that a high-fat diet enhances the formation of the types of tumors studied. This finding refers not only to the incidence of tumors but also to the average time at which they appear. As an incidental finding it appears that the spontaneous hepatoma of C3H male mice is affected by both caloric restriction and a high-fat diet, in a manner similar to the other tumors studied.

It is confirmed that the formation of tumors (incidence and average time of appearance) is dependent on the degree of caloric restriction *per se*. Evidence is presented that this is true also for diets restricted in essentials as well as calories (high or low in fat).

At a particular restricted caloric intake, a high-fat diet is less inhibitory than a diet with a low-fat content. It is also possible that a diet restricted in carbohydrate only inhibits slightly more than an equicaloric diet containing less essential components. Thus tumor formation is dependent on the *composition* of the diet, as well as on the *degree* of caloric restriction.

Evidence is presented that the enhancing effect on tumor formation of a high-fat diet, *ad libitum* or restricted, is due mainly to some specific action of fat. This action is independent of a general caloric effect that might be produced by the added consumption of the whole ration, carbohydrate, or fat.

REFERENCES

1. LAVIK, P. S., and BAUMANN, C. A. Further Studies on the Tumor-Promoting Action of Fat. Cancer Research, **3**:749-756. 1943.
2. LOEB, L., SUNTZEFF, V., BLUMENTHAL, H. T., and KIRTZ, M. M. Effect of Weight on the Development of Mammary Carcinoma in Various Strains of Mice. Arch. Path., **33**:845-865. 1942.
3. MCCAY, C. M., ELLIS, G. H., BARNES, L. L., SMITH, C. A. H., and SPERLING, GLADYS. Chemical and Pathological Changes in Aging and after Retarded Growth. J. Nutrition, **18**:15-25. 1939.
4. SAXTON, J. A. JR., BOON, M. C., and FURTH, J. Observations on the Inhibition of Development of Spontaneous Leukemia in Mice by Underfeeding. Cancer Research, **4**:401-409. 1944.

5. TANNENBAUM, A. The Initiation and Growth of Tumors. Introduction. I. Effects of Underfeeding. Am. J. Cancer, **38**:335-350. 1940.
6. TANNENBAUM, A. Relationship of Body Weight to Cancer Incidence. Arch. Path., **30**:509-517. 1940.
7. TANNENBAUM, A. Nutrition and Cancer. Bull. Am. Soc. Control Cancer, **23**:3-4. 1941.
8. TANNENBAUM, A. The Genesis and Growth of Tumors. II. Effects of Caloric Restriction per se. Cancer Research, **2**:460-467. 1942.
9. TANNENBAUM, A. The Genesis and Growth of Tumors. III. Effects of a High-Fat Diet. Cancer Research, **2**: 468-475. 1942.
10. TANNENBAUM, A. The Dependence of the Genesis of Induced Skin Tumors on the Fat Content of the Diet during Different Stages of Carcinogenesis. Cancer Research, **4**:683-687. 1944.
11. TANNENBAUM, A. The Dependence of Tumor Formation on the Degree of Caloric Restriction. Cancer Research, **5**:609-615. 1945.
12. VISSCHER, M. B., BALL, Z. B., BARNES, R. H., and SIVERTSEN, I. The Influence of Caloric Restriction upon the Incidence of Spontaneous Mammary Carcinoma in Mice. Surgery, **11**:48-55. 1942.
13. WHITE, FLORENCE R., WHITE, J., MIDER, G. B., KELLY, MARGARET, G., and HESTON, W. E. Effect of Caloric Restriction on Mammary-Tumor Formation in Strain C3H Mice and on the Response of Strain dba to Painting with Methylcholanthrene. J. Nat. Cancer Inst., **5**:43-48. 1944.

Glyoxalase Activity of Liver from Rats Fed *p*-Dimethylaminoazobenzene*

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The enzyme glyoxalase catalyzes the reaction:

$$\text{A}\quad \text{RCOCHO} \xrightarrow[H_2O]{GSH} \text{RCHOHCOOH}$$

Reduced glutathione (GSH) is required as coenzyme (12). Since Reaction A is catalyzed at a rate that, within certain limits, is independent of the substrate concentration (18), it belongs to the class of "zero order" reactions, of which the succinoxidase system is a type. Glyoxalase is found in practically all tissues, and in the case of animal tissues it is present in especially high concentrations in liver, kidney, and erythrocytes (2, 19).

Notwithstanding the features mentioned above the glyoxalase system has as yet no known function in our schemes of intermediary metabolism. For a long time it was thought that this system was important in glycolysis by serving to convert methylglyoxal, formed presumably from hexosediphosphate, to lactic acid. However, with the following observations, the role of the glyoxalase system has become obscure:

(a) both *d*(−) and *l*(+) lactic acid are formed from methylglyoxal (1),

(b) glycolysis proceeds in the absence of reduced glutathione (13),

(c) no correlation could be established between the glycolytic and glyoxalase activity of a given tissue (19).

While these facts do not necessarily rule out the possibility that the glyoxalase system represents an independent glycolytic pathway, the proof that such is actually the case is as yet not at hand.

The possibility that the glyoxalase system plays a role in intermediary metabolism other than in glycolysis is even less certain. The fact that it is not yet established with certainty that glyoxals, the substrates of this enzyme system, are naturally occurring metabolic intermediates makes the task of assigning a metabolic role to this system even more difficult. While glyoxals are highly reactive compounds capable of reacting with amino acids (15) and keto acids (8, 9, 16), these reactions do not require catalysis by the glyoxalase system. The possibility remains that glyoxals are toxic metabolic intermediates, which must be detoxified by conversion to hydroxy acids by means of the glyoxalase system. Thus methylglyoxal in concentrations of 0.01 *M* to 0.02 *M* inhibits the oxygen consumption of tissue slices (11), and when injected into rabbits produces convulsions simulating insulin shock (5). This latter effect, however, is probably due to the rapid formation of lactic acid (21). The explanation that the function of glyoxalase is that of catalyzing a detoxification reaction is obviously unsatisfactory.

Warburg, Posener, and Negelein (22) reported that slices of Flexner-Jobling rat carcinoma showed as high a glyoxalase activity as rat liver slices, while Quastel (10) found that slices of Jensen's rat sarcoma were as active as guinea pig liver slices. Platt and Schroeder (19) carried out a careful investigation of the glyoxalase activity of slices and extracts of various normal and tumor tissues. They found that the glyoxalase activity of the Walker carcinosarcoma 256 and the Philadelphia sarcoma No. 1 was of about the same order of magnitude as that found in striated muscle, but considerably lower than that found in liver. Unfortunately, a true comparison of the glyoxalase activity of normal and tumor tissue is not possible in any of these studies, since the normal tissues investigated were not histologically comparable with the tumors. However, of particular interest was the observation by Platt and Schroeder that livers from animals bearing the Walker carcinosarcoma 256 tended to have lower glyoxalase values than those from normal animals. Livers from animals bearing the Philadelphia sarcoma No. 1 were within normal limits.

Since feeding *p*-dimethylaminoazobenzene to rats results in the formation of liver tumors, it seemed of interest to measure the glyoxalase activity of livers from animals fed this compound for varying lengths of time and to compare these values with those of normal and regenerating liver.

* This investigation was aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

EXPERIMENTAL METHODS AND PROCEDURES

Animals

White rats (Sprague-Dawley) weighing between 120 and 150 gm. each were placed on the following diet, as described by Miller and his group (14).

	Per cent
Casein	12
Cerelose	70
Salt mixture	4
Vitab *	2
Corn oil (with carcinogen)	2
Cod liver oil	1

* Vitab was supplied through the courtesy of the National Oil Products Co.

The final concentration of *p*-dimethylaminoazobenzene when present in the diet was 0.06 per cent.

The animals thrived on the diet, no deaths occurring during the course of the experiment. After varying periods of time, animals were sacrificed, bled, and liver glyoxalase activity was determined. Portions of liver were also prepared and examined microscopically.

Preparation of Methylglyoxal

Methylglyoxal was prepared by the distillation of dihydroxyacetone in the presence of aqueous sulfuric acid according to Neuberg and his associates (17). The concentration of methylglyoxal in the distillate was determined according to the method of Friedemann (4).

Preparation of Liver Extracts for Glyoxalase Determination

Liver extracts were prepared according to the method of Dickens (3). Four to 6 gm. of liver were finely minced with scissors, an equal weight of water and washed sand was added, and the whole ground in a mortar to a uniform paste. This was centrifuged and the supernatant saved. The centrifugate was then returned to the mortar, another equivalent of water added, and the suspension again triturated. After centrifugation the two supernatants were combined. Additional extraction did not significantly increase the glyoxalase yield. One-third volume of 1 *M* acetic acid-sodium acetate buffer, pH 4.2, was then added with mixing and the tubes were allowed to stand in the refrigerator for 30 minutes. After centrifugation the supernatant was decanted and neutralized with one-tenth volume of 1.3 per cent $NaHCO_3$ solution. The neutralized solution was then dialyzed in the cold for 8 hours against distilled H_2O (the water was changed after 4 hours). The dialyzed solutions were then diluted with an equal volume of 1.3 per cent $NaHCO_3$ and the glyoxalase activity was determined manometrically.

Nitrogen Content of Liver Extracts

The nitrogen content of the liver extracts was determined by the micro Kjeldahl technic. The mgm. of nitrogen per ml. of enzyme solution (extract diluted with 1 part of 1.3 per cent $NaHCO_3$ solution) of the different samples are listed in Table I. On the whole there was less nitrogen extracted from the livers of animals fed with the carcinogen. This appeared to be related to the degree of cirrhotic change in these livers.

Table I: Glyoxalase Activity of Livers from Animals Fed *p*-Dimethylaminoazobenzene

Animal	Days on diet	Mgm. N in enzyme sol.	$\mu l CO_2$ produced in 20 min.	Glyoxalase units	Malignant changes
1N	...	1.10	298	27.2	0
2N	...	0.94	236	25.1	0
3N	...	1.03	252	24.5	0
4N	...	1.00	240	24.0	0
5N	...	1.20	279	23.2	0
15	94	0.80	140	17.5	0
17	65	0.81	130	16.0	0
1	58	1.12	138	15.5	0
31	110	0.80	120	15.0	+
35	110	0.85	123	14.5	0
3	78	0.85	120	14.1	0
14	110	0.90	126	14.0	++
5	124	1.02	135	13.2	+
27	124	0.78	102	13.1	++
33	78	0.85	102	12.0	+ ?
19	110	0.96	110	11.5	+
23	115	0.83	68	8.2	++
40	150	1.04	59	5.7	+++
11	145	0.78	38	4.9	++++
30	190	0.52	21	4.0	++++
29	140	0.84	31	3.7	++++
13	190	0.60	20	3.3	++++
26	145	0.95	27	2.8	++++
18	133	0.71	20	2.8	+++
12	190	0.56	14	2.5	++++
41	175	1.10	28	2.5	++++
28	200	0.80	110	13.7	+
28	200	0.56	40	7.1	+++

Manometric Determination

The main compartment of the experimental Warburg flasks contained 1 ml. of methylglyoxal solution (1.2 mgm.), 0.5 ml. of glutathione solution (0.1 mgm.), and 0.5 ml. of 1.3 per cent sodium bicarbonate solution. The side arm contained 1 ml. of glyoxalase solution (1 part extract plus 1 part 1.3 per cent sodium bicarbonate solution). The control vessels were made up in the same way except that no glutathione was present, this being replaced by 0.5 ml. of 1.3 per cent sodium bicarbonate solution. The flasks and manometers were gassed outside the bath with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide, and shaken in the bath at 26° C. The fluid levels of the manometers were adjusted to give maximum scale readings by removing a small amount of gas

after temperature equilibration, which was usually attained after shaking for 10 minutes. A zero reading was taken at this time. The contents of the side arm were then delivered into the main compartment and thoroughly mixed by swirling and pouring the solution back and forth several times. Readings were then taken every 5 minutes, while the manometers were shaking (the manometers were shaken in a horizontal position). The first 5 minute reading was usually large owing to mixing effects and was disregarded. The successive readings were plotted and the resulting curves extrapolated back to zero time. The rate of CO_2 production was remarkably constant up to the point of practically complete conversion of methylglyoxal to lactic acid. With the concentrations of methylglyoxal and glutathione used in this study it was found that the rate of CO_2 production was directly proportional to the enzyme concentration.

Glyoxalase Unit

A glyoxalase unit is defined as the amount of enzyme that will cause the production of 10 microliters of CO_2 per mgm. N in 20 minutes from 5.5×10^{-3} *M* methylglyoxal and 1.2×10^{-4} *M* glutathione at pH 7.2 and 26° C. The choice of a 20 minute period was determined by the fact that up to this time interval the rates of CO_2 production were still linear in all the experiments. A shorter period had the disadvantage that the amounts of CO_2 formed with most of the hepatoma extracts were so small during the first 10 minutes that an accurate estimation of rates was made difficult.

RESULTS

It is apparent from Table I that as the extent of neoplastic change increases there is a notable decrease in glyoxalase activity to about 10 per cent of that observed with normal liver. During the period of *p*-dimethylaminoazobenzene feeding preceding neoplastic change, the glyoxalase activity is about one-half that found in normal liver. During this latter period the microscopic changes are chiefly those of cirrhosis to a varying degree, with large numbers of injured liver cells. Since the glyoxalase activity was measured on the basis of extractable nondialyzable nitrogen, the decreased activity cannot be due to an increase in stroma or other metabolically inert tissue. In one experiment (animal 28, Table I) the macroscopically normal looking liver tissue was separated and its glyoxalase activity compared with that of the large tumor, which involved chiefly a single lobe. The difference in glyoxalase activity was not so great as might have been predicted from the gross appearance of the two parts of the same liver. Microscopically, the portion that appeared more or less normal upon gross examination showed very little cirrhosis, with occasional nests of neoplastic cells. The portion that in the gross was obviously neoplastic showed a considerable amount of neoplastic change with a great deal of cirrhosis. Unfortunately, this type of comparative study was not carried out with more animals. However, it is suggested from the data in Table I that the greatest decrease in glyoxalase activity was associated with the greatest increase in malignant tissue. The low glyoxalase activity of hepatoma tissue is particularly apparent from the values obtained with the transplants (Table II). Although these transplanted tumors had varying amounts of connective tissue, they were made up chiefly of hepatoma cells.

Table II: Glyoxalase Activity of Liver and Transplanted Hepatoma from Same Animal

Tissue	Mgm. N in enzyme sol.	$\mu l CO_2$ produced in 20 min.	Glyoxalase units
Liver	0.95	200	21.0
Hepatoma	0.52	18	3.5
Liver	0.96	223	23.2
Hepatoma	0.65	24	3.7

Table III: Glyoxalase Activity of Regenerating Liver

Liver tissue	Mgm. N in enzyme sol.	$\mu l CO_2$ produced in 20 min.	Glyoxalase units
Normal	1.12	272	24.3
Regenerating	0.85	195	22.8
Normal	0.93	218	23.5
Regenerating	0.95	214	22.5

As can be seen from Table II the livers from the host animals showed no decrease in glyoxalase activity. The tendency to a low liver glyoxalase content in rats and mice bearing carcinomas observed by Platt and Schroeder (19) apparently does not obtain for the slowly growing hepatomas. Regenerating liver from animals 7 days after hepatectomy, which had approximately 70 per cent of their livers removed, showed a normal glyoxalase content (Table III) when compared with the liver removed at the time of hepatectomy.

DISCUSSION

In using an extraction technic for enzyme assay, the question arises of the completeness of extraction of the different tissues. As previously mentioned, additional extractions of livers from both normal and carcinogen-fed rats resulted in no significant increase in glyoxalase activity of the extracts. Further, normal livers revealed a rather narrow range of assay values, both with different animals and, in a few instances, with duplicates from the same animal. Consequently

it seems reasonable to accept this extraction procedure as capable of revealing quantitative differences in glyoxalase activity of the different tissues studied.

Since the protein component of the glyoxalase system is soluble and apparently quantitatively extractable, this technic seemed more desirable than the simpler homogenization method. The value of the latter, particularly when dealing with insoluble enzyme systems, has been pointed out by Potter (20). However, in attempting to compare by the homogenization technic the relative enzyme activities of tissues with varying proportions of parenchyma and stroma, one is faced with the uncertain effect of varying amounts of cirrhosis. Since with continued feeding of *p*-dimethylaminoazobenzene an increasing percentage of the liver is represented by metabolically inert connective tissue, one could predict on a per gram whole liver basis a decrease in enzyme activity. It was thus felt that this dilemma was avoided by using an extraction method that was uniform and apparently quantitative. Further, the expression of glyoxalase activity in terms of extractable protein would appear to provide a better picture of the variation of the glyoxalase-protein in terms of the total soluble proteins of the metabolically active cells.

The significance of a decreasing glyoxalase activity in liver following *p*-dimethylaminoazobenzene feeding is not immediately apparent. While certain of the metabolic intermediates of this compound are known to react with sulfhydryl groups because of the potential quinoid structure of the former, it is not certain that the protein component of the glyoxalase system requires free sulfhydryl groups. Greenstein (6) found a 25 per cent decrease in free sulfhydryl groups in *p*-dimethylaminoazobenzene hepatomas as compared with normal liver. Part of this decrease was probably due to a decrease in glutathione. The concentration of the latter, however, is not of direct concern in the glyoxalase assay method used in the present study since the extracts were freed of glutathione by dialysis, after which the concentration was adjusted to a given level by addition.

While it is possible to speculate on the significance of a decreasing glyoxalase activity in liver from animals fed *p*-dimethylaminoazobenzene, particularly as it might relate to protein synthesis, it does not seem indicated at this time. The fact that it still remains uncertain whether glyoxals function as metabolites in normal or pathological tissues would make a discussion of the significance of varying concentrations of glyoxalase unprofitable. Perhaps of greater significance for our present understanding of the mechanism or mechanisms of carcinogenesis is the fact that the findings of this study represent another instance of a decrease in the catalytic activity or actual concentration of one of the liver proteins in the process of neoplasia (7). The fact that this occurs in the face of an increased rate of protein synthesis and without a decrease in the total protein concentration is of fundamental importance.

SUMMARY

1. Livers from rats fed *p*-dimethylaminoazobenzene for a period up to 200 days showed a decreasing glyoxalase activity. The resulting liver tumors and transplants thereof showed about 10 per cent of the glyoxalase activity of normal liver.

2. The glyoxalase activity of regenerating rat liver showed no significant change from normal.

REFERENCES

1. Dakin, H. D., and Dudley, H. W. On Glyoxalase. J. Biol. Chem., **14**:423-431. 1913.
2. Dakin, H. D., and Dudley, H. W. Glyoxalase. Part III. The Distribution of the Enzyme and its Relation to the Pancreas. J. Biol. Chem., **15**:463-474.
3. Dickens, F. Interaction of Halogenacetates and SH Compounds. The Reaction of Halogenacetic Acids with Glutathione and Cysteine. The Mechanism of Iodoacetate Poisoning of Glyoxalase. Biochem. J., **27**:1141-1151. 1933.
4. Friedemann, T. E. The Action of Alkali and Hydrogen Peroxide on Glyoxals. J. Biol. Chem., **73**:331-334. 1927.
5. Fischler, F. Beiträge zur Frage der Zuckerwirkung im Organismus. III. Mitteilung. Zur Wirkung einiger Abbauprodukte des Traubenzuckers bei Störungen des Kohlenhydratstoffwechsel. Ztschr. f. physiol. Chem., **165**:68-102. 1927.
6. Greenstein, J. P. Sulfhydryl Groups in Normal and Tumorous Hepatic Tissue Extracts before and after Addition of Salts. J. Nat. Cancer Inst., **3**:61-67. 1942.
7. Greenstein, J. P. Recent Progress in Tumor Enzymology. *In* Advances in Enzymology, Volume 3. New York: Interscience Publishers, Inc. 1943, pp. 315-348.
8. Henze, M. Die Umwandlung der Acetessigsäure durch Methylglyoxal. I. Mitteilung. Ztschr. f. physiol. Chem., **189**:121-126. 1930.
9. Henze, M., and Müller, R. Die Umwandlung der Acetessigsäure durch Methylglyoxal. II. Mitteilung. Ztschr. f. physiol. Chem., **193**:88-96. 1930.
10. Jowett, M., and Quastel, J. H. The Glyoxalase Activity of Tissues. Biochem. J., **28**:162-172. 1934.
11. Kisch, B. Beeinflussung der Gewebsatmung durch Methylglyoxal. Biochem. Ztschr., **253**:373-376. 1932.
12. Lohmann, K. Beitrag zur enzymatischen Umwandlung von synthetischen Methylglyoxal in Milchsäure. Biochem. Ztschr., **254**:332-354. 1932.
13. Lohmann, K. Über die Monojodessigsäurevergiftung des Milchsäure bildenden Ferments und der Methylglyoxalase. Biochem. Ztschr., **262**:152-156. 1933.
14. Miller, J. A., Miner, D. L., Rusch, H. P., and Baumann, C. A. Diet and Hepatic Tumor Formation. Cancer Research, **1**:699-708. 1941.
15. Neuberg, C., and Kobel, Maria. Quantitative Untersuchungen über den Abbau von Aminosäure und Aminopurinen durch Methylglyoxal und verwandte Substanzen. Biochem. Ztschr., **188**:197-210. 1927.
16. Neuberg, C., and Burkard, J. Beobachtungen über die

momentane Reaktion des Methylglyoxals mit stickstoff-freien Verbindungen. Biochem. Ztschr., **253**:373-376. 1932.

17. Neuberg, C., Färber, E., Levite, A., and Schwenk, E. Über die Hexosediphosphorsäure, ihre Zusammensetzung und die Frage ihrer Rolle bei der alkoholischen Gärung sowie über das Verhalten der Dreikohlenstoffzucker zu Hefen. Biochem. Ztschr., **83**:244-268. 1917.
18. Petrovicki, H. Kinetische Untersuchungen an der Methylglyoxalase. Biochem. Ztschr., **303**:186-199. 1939.
19. Platt, Muriel E., and Schroeder, E. F. Glyoxalase. II. The Distribution of Glyoxalase in Tissues of Normal and Cancerous Albino Rats. J. Biol. Chem., **106**:179-190. 1934.
20. Potter, V. R. Biological Energy Transformations in the Cancer Problem. *In* Advances in Enzymology, Volume 4. New York: Interscience Publishers, Inc. 1944, pp. 201-256.
21. Schneider, E., and Widmann, E. Untersuchungen über den Kohlenhydratumsatz und den Abbau des Traubenzuckermoleküls. Klin. Wchnschr., **8**:536-541. 1929.
22. Warburg, O., Posener, K., and Negelein, E. Über den Stoffwechsel der Carcinomzelle. Biochem. Ztschr., **152**: 309-344. 1924.

Glyoxalase Activity of Erythrocytes from Cancerous Rats and Human Subjects*

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The demonstration of a striking decrease in glyoxalase activity of liver from rats fed *p*-dimethylaminoazobenzene (1) suggested the possibility that other tissues rich in glyoxalase might be similarly affected. Since the glyoxalase activity of erythrocytes is very high (2, 3) it seemed of interest to determine the activity of this enzyme system in rats fed the compound just mentioned. Since a previous study (1) suggested that the greatest decrease in glyoxalase activity was associated with the extent of neoplasia, erythrocytes from human subjects with and without malignant neoplastic disease were also studied. While no significant variation from the normal was observed, some points of interest are suggested by the findings.

METHODS

Preparation of Erythrocytes

The glyoxalase activity of erythrocytes was estimated after hemolysis, using essentially the technic of Quastel (3). Blood was collected in 2 per cent sodium citrate solution (0.18 ml. per 1 ml. of blood). An aliquot was taken for hematocrit determination. After centrifugation and removal of the supernatant layer, the cells were suspended in saline and again centrifuged down. The supernatant layer was again decanted and 9 volumes of distilled water were added to lyse the cells. An aliquot of the 1:10 solution was then diluted with 4 volumes of 1.3 per cent sodium bicarbonate solution to give a final dilution of 1:50.

Estimation of Glyoxalase Activity

0.5 ml. of the 1:50 solution of lysed erythrocytes was placed in the side arm of Warburg flasks. The main compartment contained 1 ml. of methylglyoxal solution (1.2 mgm.) and 0.5 ml. of glutathione solution (2.0 mgm.) The final concentration of the former was 0.0083 *M* and of the latter 0.0035 *M*. This concentration of glutathione was established to be the optimum for the experimental conditions employed. Control flasks contained 0.5 ml. of 1.3 per cent sodium bicarbonate solution in place of the glutathione. All experiments were run anaerobically, the flasks being filled with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. The manometric procedures were otherwise the same as those employed for the estimation of liver glyoxalase activity (1). The rates of carbon dioxide production were found to be linear for 20 to 30 minutes, after which the rates started to fall off. The first 5 minute reading was disregarded. Glyoxalase activity is expressed as the microliters of carbon dioxide produced in 20 minutes. Approximately 50 per cent of the methylglyoxal was converted to lactic acid during this interval.

RESULTS

The glyoxalase activity of erythrocytes from normal and tumor-bearing rats is shown in Table I. As can be seen from the table, the glyoxalase activity of erythrocytes from the tumor-bearing animals falls within the same range as that found with normal animals. The animals with induced tumors had all been on a *p*-dimethylaminoazobenzene diet for 190 days, and the growths were all large. The transplanted neoplasms were large also, and had undergone some degeneration; the animals bearing them were in particularly bad shape, which probably accounts for the extremely low hematocrit values found in this group.

Table I: Glyoxalase Activity of Erythrocytes from Normal and Tumor-Bearing Rats

Animal	Hematocrit	$\mu l CO_2$ formed in 20 min. per 0.01 ml. R.B.C.	$\mu l CO_2$ formed in 20 min. per 0.01 ml. whole blood
Normal	45.0	204	92.0
"	42.5	198	84.2
"	43.0	210	90.3
"	39.1	202	79.0
Hepatoma	34.2	208	71.2
"	39.3	185	72.5
"	30.2	201	60.8
Transplanted hepatoma	25.6	208	53.3
" "	28.9	190	55.0

The glyoxalase activity of erythrocytes from normal human subjects and from patients with neoplastic and

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other diseases is shown in Table II. Here, again, the glyoxalase activity of the erythrocytes shows no striking variation from the normal range.

In both rat and man the glyoxalase activity, expressed on the basis of whole blood, shows considerable variation due to the variation in hematocrit values.

TABLE II: GLYOXALASE ACTIVITY OF ERYTHROCYTES FROM HUMAN SUBJECTS

Diagnosis	Hematocrit	$\mu l CO_2$ formed in 20 min. per 0.01 ml. R.B.C.	$\mu l CO_2$ formed in 20 min. per 0.01 ml. whole blood
Normal	45.8	146	67.0
"	41.6	144	60.0
"	48.5	138	67.0
"	39.5	150	59.3
"	41.3	140	57.7
"	49.2	132	65.0
"	51.0	138	70.5
"	46.5	135	63.0
"	42.5	142	42.5
"	44.8	133	59.6
Carcinoma (face)	45.2	138	62.5
" (colon)	41.8	134	56.0
" (bile duct)	39.6	134	53.2
" (lung)	40.5	138	56.0
" (pancreas)	37.3	126	47.0
" (breast)	39.8	142	56.5
" "	36.3	128	45.3
" (stomach)	44.5	146	65.0
" (rectum)	38.7	140	54.2
" "	45.3	130	59.0
Leukemia, acute	26.9	134	36.0
" chronic	35.3	140	48.4
Polycythemia vera	65.1	138	90.0
Pernicious anemia	40.5	126	51.0
" "	38.5	138	53.2
Obstructive jaundice	36.1	126	45.5

DISCUSSION

The failure to find any significant difference in the glyoxalase activity or erythrocytes from cancer patients as compared with normal persons is perhaps not surprising. On the other hand, the finding of normal values for erythrocytes from animals with *p*-dimethylaminoazobenzene tumors was somewhat unexpected. These animals had been on the carcinogenic diet for 190 days and the glyoxalase activity of their livers was notably reduced as compared with normal. This finding suggests that either the metabolic intermediates of *p*-dimethylaminoazobenzene do not reach the blood stream in sufficient concentration to affect the intracellular contents of the erythrocyte, or that the decrease in glyoxalase activity observed in the livers of these animals is secondary to, or concurrent with, the neoplastic changes. Evidence against the former possibility is seen in the study of Miller and his associates (4), where it was demonstrated that certain of the *p*-dimethylaminoazobenzene intermediates, chiefly *p*-aminoazobenzene, were present in higher concentration within the erythrocyte than in any other tissue of the rat. It would thus appear that the decrease in glyoxalase activity in livers from rats fed this carcinogen is not due directly to the influence of this substance or its metabolic intermediates.

The physiological significance of a decreased glyoxalase activity on a whole blood basis as seen in cases with low hematocrit values is difficult to assess. In all probability this is without metabolic significance. Until the metabolic role of the glyoxalase system and the glyoxals are more clearly defined, the significance of such findings will continue to remain obscure.

SUMMARY

Erythrocytes from patients with neoplastic and other diseases, and erythrocytes from rats with *p*-dimethylaminoazobenzene hepatomas and transplanted hepatomas showed no significant change in glyoxalase activity as compared with erythrocytes from normal subjects.

REFERENCES

1. COHEN, P. P. Glyoxalase Activity of Liver from Rats Fed *p*-Dimethylaminoazobenzene. Cancer Research. **5**:626-630. 1945.
2. DAKIN, H. D., and DUDLEY, H. W. Glyoxalase. Part III. Distribution of the Enzyme and its Relation to the Pancreas. J. Biol. Chem., **15**:463-474. 1913.
3. JOWETT, M., and QUASTEL, J. H. The Glyoxalase Activity of the Red Blood Cell. The Function of Glutathione. Biochem. J., **27**:486-498. 1933.
4. MILLER, J. A., MILLER, E. C., and BAUMANN, C. A. On the Methylation and Demethylation of Certain Carcinogenic Azo Dyes in the Rat. Cancer Research, **5**:162-168. 1945.

The Biological Assay of Carcinogens

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Many experiments have been described in which a batch of animals are painted or injected with a carcinogen, and the dates recorded when warts or malignant growths are detected. The object of such experiments is usually to compare the potency of different carcinogens, or of different doses or methods of application, or else to compare the sensitivity of different batches of animals to a given carcinogen. It is convenient to have some means of summarizing the results of each experiment in a single number, together with an indication of its precision. The purpose of the present paper is to describe suitable procedures for arriving at such a number. The methods described are not essentially new; in particular the procedures advocated by C. I. Bliss (4) in a paper on the calculation of the time-mortality curve are in the main applicable to the present problem, and the method given below for dealing with experiments in which some animals die prematurely without tumor is an adaptation of a method described by W. L. Stevens (17) in an appendix to the Bliss paper.

EXPERIMENTS IN WHICH ALL THE ANIMALS DEVELOP TUMORS

We take first the case of experiments in which all the animals that are treated get tumors. The most obvious procedure in such cases would be to average the times (after the beginning of treatment) at which tumors were recorded, and to take this mean time as a measure of the effectiveness of the treatment employed (the shorter the time, the more effective the treatment). The precision of the mean would be expressed by calculating its standard deviation in the usual fashion.

Calculating the arithmetic mean would be the best method of summarizing the observations if the individual induction times were distributed in a normal (Gaussian) distribution. The arithmetic mean, however, is not necessarily the best way of combining observations not distributed normally, and it is in fact possible to imagine distributions in which the arithmetic mean of many observations is of not much higher precision than a single observation (11, 12). It is necessary, therefore, to consider the manner in which the times when tumors are recorded are distributed among the individual animals of a batch. Fig. 1, A, is a histogram showing the number of mice developing tumors in successive fortnightly periods following a single injection of 0.5 mgm. of methylcholanthrene into each of 86 mice (Shimkin and Andervont [15], Series A, C, and D grouped together). The distribution is evidently not symmetrical. An alternative method of graphical representation is to plot against the time after injection the percentage of animals that acquired tumors up to that time. The method is shown in B, Fig. 1; the curve is not symmetrical about the ordinate erected at 50 per cent. A more sensitive method of testing graphically whether a distribution is normal or not is to plot the percentages on probability paper as in C, Fig. 1. A systematic departure of the points from a straight line, such as is evident here, indicates a non-normal distribution. As an alternative to the use of probability paper, the same effect may be achieved by converting the percentages of animals with tumors into *probits* by using the tables prepared by Bliss (3) (reproduced also in Fisher and Yates' Statistical Tables [13]). This procedure is illustrated in D, Fig. 1. It is necessary to omit points at 0 per cent and 100 per cent when plotting data in the manner of C or D, Fig. 1.

If we take as a measure of the time after treatment at which a tumor is recorded, not the time in weeks but its logarithm (to base 10), then it appears that the distribution is much more nearly normal. In E, Fig. 1, the histogram shows the number of animals developing tumors in the successive intervals of time whose logarithms are 0.80—0.85, 0.85—0.90, 0.90—0.95 etc., *i.e.* in time intervals 6.31—7.08, 7.08—7.94, 7.94—8.91, etc. weeks. Fig. 1, F, G, and H, show the forms taken by Fig. 1, B, C, D, when the logarithm of the time in weeks, instead of the time itself, is plotted as abscissa. The fact that the points lie fairly well on straight lines in Fig. 1, G and H, shows that the logarithm of the induction time is distributed approximately normally.

While the normal distribution of the logarithms of the time of induction has no theoretical basis, empirically it seems to be of wide validity, as is shown by the curves A to E of Fig. 2, in which 5 further

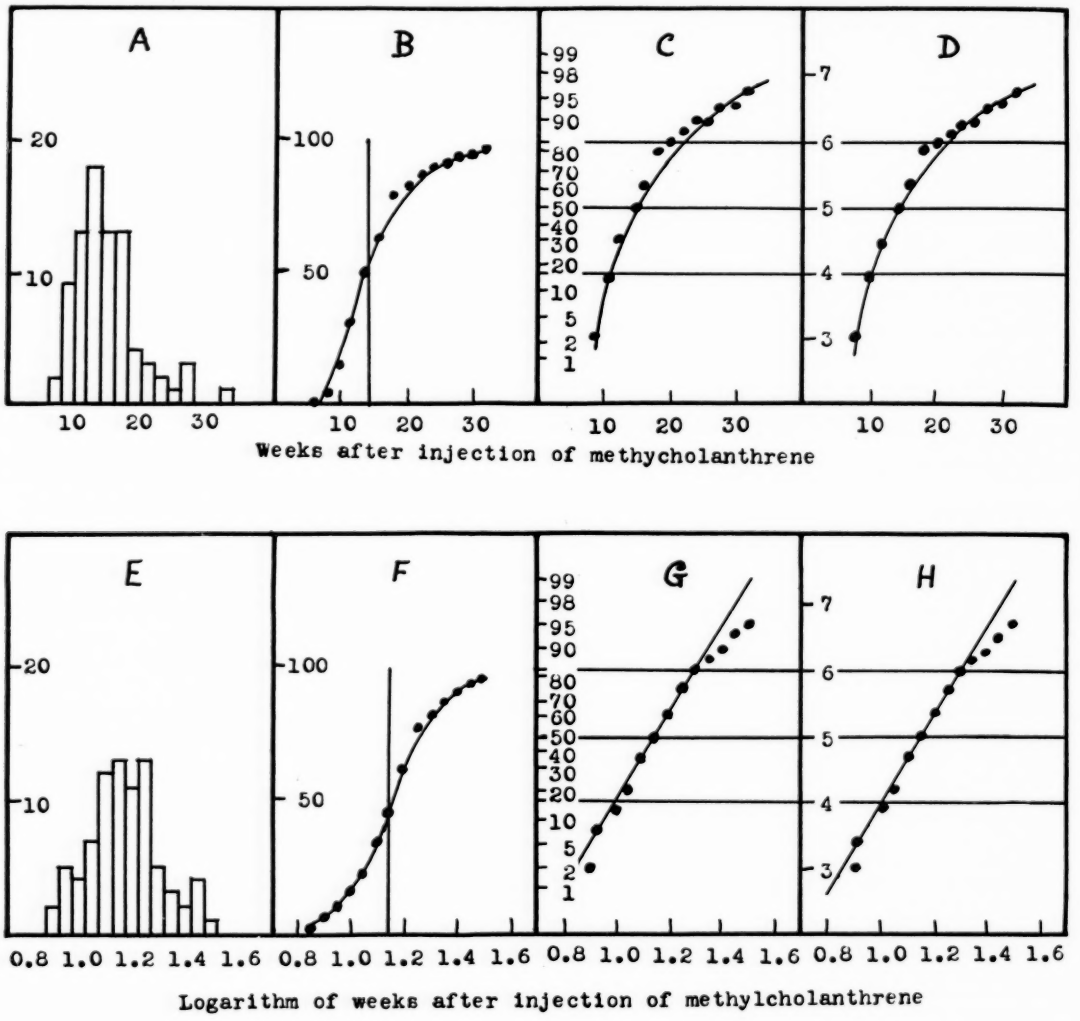

FIG. 1.—Graphical methods of representation. (Experiments of Shimkin and Andervont [15]. For explanation, see text.)

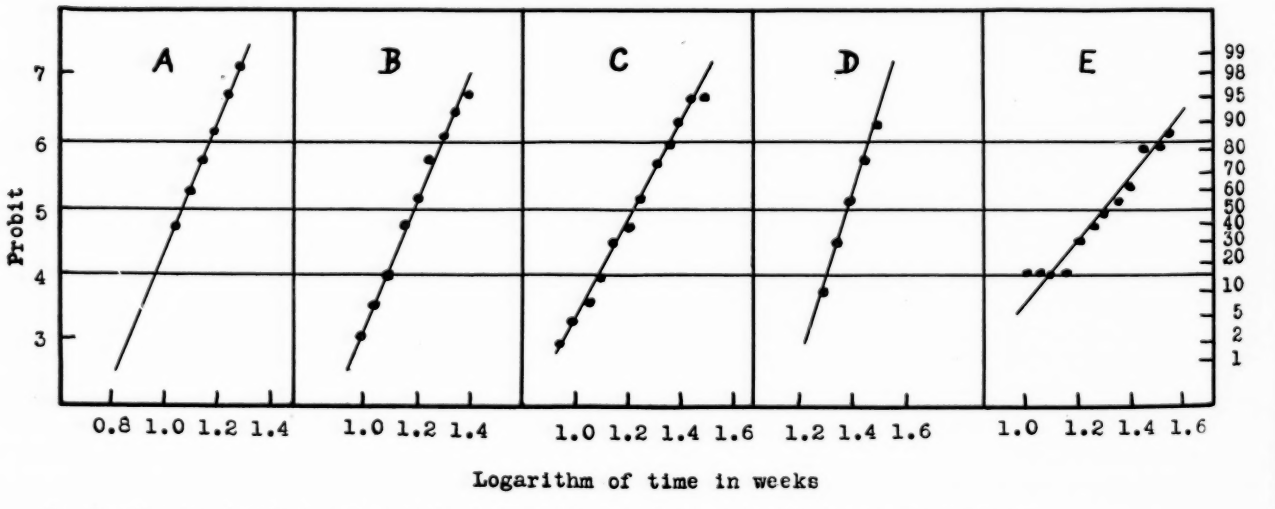

FIG. 2.—Showing that the logarithm of the time of induction of tumors in mice is normally distributed.

A. Single subcutaneous injection of benzpyrene (Shimkin and Andervont [16], Table I. Data for 0.5, 1.0, 2.0 mgm. of benzpyrene have been combined).

B. Thrice-weekly paintings with methylcholanthrene in acetone (Stowell and Cramer [18]).

C. Weekly paintings with tar (Bonser [7], strain JCF1).

D. Paintings with benzpyrene in benzene at 8-day intervals for 6 months (Beck and Peacock [2], Group 7; papillomas).

E. Single subcutaneous injection of methylcholanthrene (Andervont and Shear [1], pellet removed after 6 weeks).

sets of data are plotted in the manner of G and H, Fig. 1; *i.e.*, as abscissa is taken the logarithm of the time in weeks, and as ordinate either the percentage is plotted on probability paper (*cp.* right hand ordinate scale of Fig. 2) or the percentage is converted to a probit and the probit plotted on ordinary graph paper (*cp.* left hand ordinate scale of Fig. 2). It has been found that the logarithm of the time of appearance of spontaneous lung tumors, and of skin tumors induced by ultraviolet light, is normally distributed (5, 6).

Since the logarithm of the time of induction is normally distributed, to calculate the mean of the logarithms is a satisfactory method of combining the data. The standard deviation of the distribution of log times is calculated in the usual fashion as

$$s = \sqrt{\frac{S(t-m)^2}{n-1}},$$

where the numerator is the sum of the squares of the deviations $(t-m)$ between the logarithm (t) of the induction time and the mean logarithm (m), and $n-1$ is one less than the number of tumors n. The standard error to be attached to the mean logarithm is then $s/\sqrt{n}$.

Graphically, the mean m may be read off as the abscissa of the point where a straight line drawn through the experimental points plotted as in Fig. 2 passes through a horizontal line at the 50 per cent level (probit 5), while the standard deviation s may be read off as the interval between this abscissa and the abscissa for the intersection with a horizontal line at the 84 per cent level (probit 6) or the 16 per cent level (probit 4). The arithmetical computation is to be preferred to the graphical.

The antilogarithm of the mean log induction time is the geometrical mean of the experimental induction times. There is no necessity, however, to take the antilogarithm, and tests of the significance of the difference between two treatments will be made by comparing the mean log induction times.

The relation between the mean and variance of the times, and the mean and variance of the log times in a distribution of this sort has been discussed (10).

The antilogarithm of the mean log induction time will not differ systematically from the time at which 50 per cent of the animals get tumors (since the mean and median of a normal distribution coincide), and it might be thought that to determine the time at which 50 per cent of the animals in the experiment get tumors would be a simpler procedure. However, it is known that the precision of the estimate obtained in this way is less than the precision of the estimate obtained in the manner recommended above, and its use is equivalent to wasting one-third of the observations (12).

Ideally, to obtain the maximum amount of information from a given number of animals, the time at which a tumor is detectable should be recorded as accurately as possible, necessitating frequent examination. In practice the loss of precision in making less frequent observations is not very great, and is repaid by the saving of labor. Where the experiment involves painting at weekly or more frequent intervals, the examination for tumors will usually be made at these times. Where a single injection of a carcinogen has been given, and the experiment does not therefore necessitate the frequent handling of the animals, 10 or more examinations over the period during which tumors are appearing will generally suffice. It is desirable, however, to choose the times so that their logarithms are equally spaced. For example, if one wished to divide the period 20 to 40 weeks after injection into 10 intervals, one would preferably not examine at 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40 weeks, but instead at 20.0, 21.4, 22.9, 24.5, 26.3, 28.2, 30.2, 32.4, 34.7, 37.2, 39.8 weeks (*i.e.* 140, 150, 160, 172, 184, 197, 211, 227, 243, 260, 279 days), these being the antilogarithms of the numbers 1.30, 1.33, 1.36, 1.39, 1.42, 1.45, 1.48, 1.51, 1.54, 1.57, 1.60.

EXPERIMENTS IN WHICH NOT ALL THE ANIMALS GET TUMORS

Experiments are frequently made in which, at the end of the period of observation (a practical limit to which is set by the life of the animals), some of the animals have not got tumors. The method of combining the data is necessarily more complicated than when all the animals develop tumors.

Some authors have determined the percentage of animals with tumors at the end of the experiment, and have taken this percentage as a measure of the effectiveness of the treatment. In addition they have calculated the mean time of induction of tumors in those animals in which tumors were induced, and have regarded this as a separate measure of the effectiveness of the treatment. Sometimes the two measures have been combined according to an arbitrary rule to give a "carcinogenic index" (14). Bryan and Shimkin (8, 9), in discussing the response of animals to variations in the dose of the carcinogen injected, plot the probit corresponding to the percentage of the animals getting tumors against the logarithm of the dose of carcinogen in the manner recommended by Bliss (3) for analyzing experiments on the killing of organisms by different concentrations of a lethal agent. This procedure does not, to the present author, appear to be the best way of utilizing the data in experiments on carcinogenesis.

It appears reasonable, when analyzing an experiment in which some animals are without tumors at the

end of the period of observation, to regard these animals as having an induction time longer than the duration of the experiment, and in effect to assume that had it been practicable to observe these animals for a sufficiently long time, all would have got tumors. Such an assumption is consistent with the observation that treatments that give a low percentage of tumors in general give a long mean induction time in the animals that get tumors (*cp.* data quoted by Bryan and Shimkin [8]). The problem is then to estimate the mean of a distribution when we know the induction time of some animals, and regarding others merely know that the induction time exceeds a stated figure. This problem is solvable, by appropriate methods.

The assumption that animals without tumors at the end of the experiment would get tumors if the observations could be continued for a sufficiently long time may not be a valid one if the application of the carcinogen is transitory; for example, if a few paintings are given and then discontinued. However, the assumption seems reasonable in experiments in which the painting is continued, or in which a persistent carcinogen has been injected.

It is desirable that the animals should be of an inbred strain, since the method depends on assuming a smooth distribution of induction time. This consideration applies equally to the method advocated by Bryan and Shimkin (8).

The procedure described below also enables account to be taken of animals that die without tumor before the completion of the experiment, providing the time is recorded at which observation of these animals ceased. It is in order to make provision for these animals that the procedure devised by Bliss (4) and Stevens (17) for dealing with a truncated distribution has been modified.

Principle of the Method *

The method of maximum likelihood of Fisher (11) is used, its application to the present problem being modified from Stevens (17). The logarithm of the induction time being supposed normally distributed with variance σ^2 about the value μ, the probability that an animal will acquire a tumor between log-times t and $t+dt$ is the product of the probability that it will live to a time not less than t and $z\,dt/\sigma$, where

$$z = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} \text{ and } x = (t-\mu)/\sigma.$$

Writing

$$q = \int_x^\infty z\,dx,$$

the probability that it will die without tumor between log-times t and $t+dt$ is the product of q and the probability that it will die between these times. Providing that we can assume there is no correlation between expectation of life and susceptibility to the carcinogen, the only contribution depending on μ and σ that is made to the log-likelihood (L) by an animal acquiring a tumor at log-time t is $\log(z/\sigma)$, and by an animal dying without a tumor at t is $\log q$. By differentiation we can draw up the following table (Table I) of contributions to L and its derivatives by an animal of each of the two categories.

The contributions to the quantities A, B, C, D, E made by an animal that gets a tumor at log-time t are readily calculated in terms of the corresponding values of x by use of the formulae in the center column of Table I. The contributions to the quantities A, B, C, D, E, made by an animal that dies without tumor at log-time t are given by the formulae in the right hand column of Table I, and have been tabulated for a range of values of x in Table II. A is the sum of the contributions to A made by all the animals, with and without tumors, and similarly with B, C, D, and E.

Table I

Quantity	Contribution by an animal getting a tumor at log-time t	Contribution by an animal not getting a tumor by log-time t
L	$\log(z/\sigma)$	$\log q$
$A=\sigma\frac{\partial L}{\partial \mu}$	x	$\frac{z}{q}$
$B=\sigma\frac{\partial L}{\partial \sigma}$	(x^2-1)	$\frac{xz}{q}$
$C=-\sigma^2\frac{\partial^2 L}{\partial \mu^2}$	1	$\frac{z^2}{q^2}-\frac{xz}{q}$
$D=-\sigma^2\frac{\partial^2 L}{\partial \mu \partial \sigma}$	$2x$	$\frac{z(1-x^2)}{q}+\frac{xz^2}{q^2}$
$E=-\sigma^2\frac{\partial^2 L}{\partial \sigma^2}$	$(3x^2-1)$	$\frac{xz(2-x^2)}{q}+\frac{x^2z^2}{q^2}$

According to the method of maximum likelihood, the best estimates of μ and σ to be derived from the data are the solutions of the equations $\frac{\partial L}{\partial \mu}=0$ and $\frac{\partial L}{\partial \sigma}=0$, *i.e.*, of $A=0$ and $B=0$. These solutions we shall arrive at by continued approximations. A first approximation, obtained graphically (as described later) gives, say, the estimates m_1 for μ and s_1 for σ. With these values inserted for μ and σ in the formula $x=(t-\mu)/\sigma$, each experimental log-time t can be converted into the corresponding value of x, and the quantities A, B, C, D, E evaluated. In general A and B so evaluated will not be zero. The corrections

* The reader who does not propose to follow the mathematics should resume reading at the heading *Arithmetical Procedure*.

TABLE II

x	A	B	C	D	E
−2.0	0.0552	−0.1105	0.1135	−0.1718	0.2332
−1.9	0.0676	−0.1284	0.1329	−0.1850	0.2231
−1.8	0.0819	−0.1474	0.1541	−0.1955	0.2045
−1.7	0.0984	−0.1673	0.1770	−0.2025	0.1769
−1.6	0.1174	−0.1878	0.2015	−0.2051	0.1404
−1.5	0.1388	−0.2082	0.2274	−0.2024	0.0954
−1.4	0.1629	−0.2280	0.2546	−0.1935	0.0429
−1.3	0.1897	−0.2467	0.2827	−0.1777	−0.0156
−1.2	0.2194	−0.2633	0.3115	−0.1543	−0.0781
−1.1	0.2520	−0.2773	0.3408	−0.1228	−0.1422
−1.0	0.2876	−0.2876	0.3703	−0.0827	−0.2049
−0.9	0.3261	−0.2935	0.3998	−0.0338	−0.2631
−0.8	0.3676	−0.2940	0.4292	0.0242	−0.3134
−0.7	0.4119	−0.2883	0.4580	0.0913	−0.3523
−0.6	0.4591	−0.2755	0.4863	0.1674	−0.3759
−0.5	0.5092	−0.2546	0.5138	0.2522	−0.3807
−0.4	0.5619	−0.2248	0.5405	0.3457	−0.3630
−0.3	0.6172	−0.1852	0.5661	0.4474	−0.3194
−0.2	0.6751	−0.1350	0.5907	0.5569	−0.2464
−0.1	0.7353	−0.0735	0.6142	0.6739	−0.1409
0.0	0.7979	0.0000	0.6366	0.7979	0.0000
0.05	0.8300	0.0415	0.6474	0.8624	0.0846
0.10	0.8626	0.0863	0.6578	0.9284	0.1791
0.15	0.8958	0.1344	0.6680	0.9960	0.2838
0.20	0.9294	0.1859	0.6779	1.0650	0.3989
0.25	0.9636	0.2409	0.6875	1.1354	0.5247
0.30	0.9982	0.2994	0.6969	1.2072	0.6616
0.35	1.0332	0.3616	0.7059	1.2803	0.8097
0.40	1.0688	0.4275	0.7147	1.3547	0.9694
0.45	1.1047	0.4971	0.7233	1.4302	1.1407
0.50	1.1411	0.5705	0.7315	1.5068	1.3240
0.55	1.1779	0.6478	0.7395	1.5846	1.5193
0.60	1.2150	0.7290	0.7473	1.6634	1.7270
0.65	1.2526	0.8142	0.7548	1.7432	1.9472
0.70	1.2905	0.9033	0.7620	1.8239	2.1801
0.75	1.3288	0.9966	0.7691	1.9056	2.4258
0.80	1.3674	1.0939	0.7759	1.9881	2.6844
0.85	1.4064	1.1954	0.7824	2.0714	2.9561
0.90	1.4456	1.3011	0.7888	2.1556	3.2411
0.95	1.4852	1.4110	0.7950	2.2404	3.5394
1.00	1.5251	1.5251	0.8009	2.3260	3.8512
1.05	1.5653	1.6436	0.8067	2.4123	4.1765
1.10	1.6058	1.7664	0.8122	2.4992	4.5155
1.15	1.6465	1.8935	0.8176	2.5868	4.8683
1.20	1.6876	2.0251	0.8228	2.6749	5.2349
1.25	1.7288	2.1610	0.8278	2.7635	5.6155
1.30	1.7703	2.3014	0.8326	2.8528	6.0100
1.35	1.8121	2.4463	0.8373	2.9425	6.4186
1.40	1.8541	2.5957	0.8419	3.0327	6.8414
1.45	1.8963	2.7496	0.8462	3.1233	7.2784
1.50	1.9387	2.9080	0.8505	3.2144	7.7296
1.55	1.9813	3.0710	0.8545	3.3058	8.1951
1.60	2.0241	3.2386	0.8585	3.3977	8.6750
1.65	2.0671	3.4108	0.8623	3.4900	9.1692
1.70	2.1104	3.5876	0.8660	3.5826	9.6780
1.75	2.1537	3.7691	0.8696	3.6755	10.2012
1.80	2.1973	3.9552	0.8730	3.7688	10.7389
1.85	2.2410	4.1459	0.8764	3.8623	11.2912
1.90	2.2849	4.3414	0.8796	3.9562	11.8581
1.95	2.3290	4.5416	0.8827	4.0503	12.4396
2.00	2.3732	4.7464	0.8857	4.1447	13.0357

δm to be added to m_1, and δs to be added to s_1, needed to bring A and B to zero, are evidently given by

$$0 = A + \left(\frac{\partial A}{\partial \mu}\right)_1 \delta m + \left(\frac{\partial A}{\partial \sigma}\right)_1 \delta s$$

$$0 = B + \left(\frac{\partial B}{\partial \mu}\right)_1 \delta m + \left(\frac{\partial B}{\partial \sigma}\right)_1 \delta s$$

where the suffix 1 indicates evaluation at $\mu = m_1$, $\sigma = s_1$, *i.e.*, by

$$0 = As_1 - C\delta m - D\delta s$$
$$0 = Bs_1 - D\delta m - E\delta s$$

whence

$$\delta m = s_1(AG + BH)$$
$$\delta s = s_1(AH + BJ)$$

where $F = CE - D^2$, $G = E/F$, $H = -D/F$, $J = C/F$.

If we accept the values of C, D, E, evaluated with the experimental values of log-time as sufficiently near approximations to the expectations of $-\sigma^2 \frac{\partial^2 L}{\partial \mu^2}$, $-\sigma^2 \frac{\partial^2 L}{\partial \mu \partial \sigma}$, $-\sigma^2 \frac{\partial^2 L}{\partial \sigma^2}$, then the standard deviations given by the method of maximum likelihood are,

$$\text{s.d. of } m = s\sqrt{G}, \qquad \text{s.d. of } s = s\sqrt{J}.$$

ARITHMETICAL PROCEDURE

The derivation of the mean log induction time from an experiment in which some of the animals fail to get tumors, proceeds in two stages. The first stage is to make approximate estimates m_1 and s_1 of the mean and standard deviation of the distribution of log induction times. Corrections δm and δs are then calculated, which are to be added to these first approximations to get the final values.

The first approximations m_1 and s_1 are deduced (as explained previously) from a graph drawn in the manner of Fig. 2. We illustrate the procedure by working up an experiment by Andervont and Shear, in which a pellet of 0.5 mgm. of methylcholanthrene was inserted under the skin of the abdomen and removed after 6 weeks. Twenty-five mice were treated in this fashion; 22 animals acquired tumors, the times when the tumor was first detected being recorded. One animal had no tumor when observation ceased 40 weeks after treatment. Two animals died without tumor, at 27 and 33 weeks.

For drawing the graph (E, Fig. 2) the percentage of animals with tumors at each date is calculated by dividing the number of animals getting tumors up to that date by 25 and multiplying by 100. Percentages calculated in this way are liable to be too low in the later part of the experiment, since no allowance has been made for the possibility that some of the animals that died prematurely might have got tumors had they lived longer. Various devices have been pro-

posed for reducing this error (*cp.*, for example, [8]). None of them are entirely satisfactory, but their use is permissible (though not essential) in drawing the graph, and will often give a better first approximation than the uncorrected data. The uncorrected data are, however, quite adequate in an experiment such as the the times at which the tumors appeared are converted to log times (t), m_1 is subtracted to form $(t-m_1)$ and the values of $(t-m_1)$ for all the tumors are summed to form $S(t-m_1)$. (S denotes summation.) If the data are grouped, as are those which we have worked up in Table III, the value of $(t-m_1)$ for the center of

TABLE III: DATA OF ANDERVONT AND SHEAR (1). GRAPHICAL FIRST APPROXIMATIONS (E, FIG. 2) ARE $m_1 = 1.30$; $s_1 = 0.20$

22 ANIMALS WITH TUMORS

Time	t = log-time	f = number of animals	$(t-m_1)$	$f(t-m_1)$		$(t-m_1)^2$	$f(t-m_1)^2$
9	0.95	4	−0.35	−1.40	−2.06	0.1225	0.4900
15	1.18	4	−0.12	−0.48		0.0144	0.0576
17	1.23	2	−0.07	−0.14		0.0049	0.0098
19	1.28	2	−0.02	−0.04		0.0004	0.0008
21	1.32	2	0.02	0.04	1.07	0.0004	0.0008
23	1.36	1	0.06	0.06		0.0036	0.0036
25	1.40	2	0.10	0.20		0.0100	0.0200
27	1.43	3	0.13	0.39		0.0169	0.0507
29	1.46	1	0.16	0.16		0.0256	0.0256
33	1.52	1	0.22	0.22		0.0484	0.0484

$n_1 = 22$ $\quad S(t-m_1) = -0.99$ $\quad S(t-m_1)^2 = 0.7073$

$$S\left(\frac{t-m_1}{s_1}\right) = S(x) = \frac{-0.99}{0.2} = -4.95 \qquad 2S(x) = -9.90$$

$$S\left(\frac{t-m_1}{s_1}\right)^2 = S(x^2) = \frac{0.7073}{0.04} = 17.6825$$

$S(x^2) - n_1 = 17.6825 - 22 = -4.3175$ $\quad 3S(x^2) - n_1 = 31.0475$

3 ANIMALS WITHOUT TUMORS

Time	t = log-time	$(t-m_1)$	$x = \left(\frac{t-m_1}{s_1}\right)$
27	1.43	0.13	0.65
33	1.52	0.22	1.10
40	1.60	0.30	1.50

	A	B	C	D	E
22 animals with tumors	−4.9500	−4.3175	22.0000	−9.9000	31.0475
No tumor at $x = 0.65$	1.2526	0.8142	0.7548	1.7432	1.9472
No tumor at $x = 1.10$	1.6058	1.7664	0.8122	2.4992	4.5155
No tumor at $x = 1.50$	1.9387	2.9080	0.8505	3.2144	7.7296
Totals	−0.1529	1.1711	24.4175	−2.4432	45.2398

$F = 24.4175 \times 45.2398 - (2.4432)^2 = 1098.67$

$G = 45.2398/1098.67 = 0.0412$; $\quad H = 2.4432/1098.67 = 0.0022$;

$J = 24.4175/1098.67 = 0.0222$

$\delta m = 0.2(-0.1529 \times 0.0412 + 1.1711 \times 0.0022) = -0.0007 \quad \therefore m = 1.299$

$\delta s = 0.2(-0.1529 \times 0.0022 + 1.1711 \times 0.0222) = +0.0051 \quad \therefore s = 0.205$

$s\sqrt{G} = 0.205 \times 0.203 = 0.0416$

Mean log induction time is 1.299 ± 0.042

present one, in which not many animals die without tumor before the end of the experiment.

Andervont and Shear present their data as numbers of tumors appearing in successive fortnightly intervals; the data have been regrouped in drawing E, Fig. 2, into equal increments 0.05 of log-time. From the graph we read off $m_1 = 1.30$, $s_1 = 0.20$.

Dealing first with the n_1 animals that got tumors, each group is multiplied by the number (f) of animals in the group. Dividing by s_1 gives $S(x)$, where $x = (t-m_1)/s_1$. The values of $(t-m_1)$ are now squared, summed in the same way, and divided by s_1^2 to give $S(x^2)$. A table is now drawn up with 5 columns, headed *A, B, C, D, E,* into which are entered respectively $S(x)$; $S(x^2)-n_1$; n_1; $2S(x)$; $3S(x^2)-n_1$.

The animals without tumors are next dealt with.

The times at which observation on the individual animals ceased are converted into log-times (t), m_1 is subtracted to give $t-m_1$, and this is divided by s_1 to give $x=(t-m_1)/s_1$. For each value of x we read off from Table II numbers to enter under the column headings *A, B, C, D, E*. In using Table II it is satisfactory to use the tabulated value of x nearest to the value of $(t-m_1)/s_1$; interpolation is unnecessary. On the infrequent occasions on which a value of x outside the range of the table is met with, the following procedure may be adopted without great error. If x is less than -2.0, the animal is neglected completely. If x exceeds $+2.0$, the animal concerned is treated as if it developed a tumor at the last time at which it was observed.

The values of *A, B, C, D, E* are now obtained by summing the contributions that have been written down, for all the animals with and without tumors. From *C, D, E* we derive the following numbers:

$$F=CE-D^2; \quad G=E/F; \quad H=-D/F; \quad J=C/F.$$

Finally the corrections δm to be added to m_1, and δs to be added to s_1 are calculated by the formulas,

$$\delta m=s_1(AG+BH); \quad \delta s=s_1(AH+BJ)$$

and the final values are:

$$m=m_1+\delta m; \quad s=s_1+\delta s.$$

The standard deviation to attach to m is $s\sqrt{G}$.

The corrections δm and δs in the example worked out in Table III are very small, owing to the fortuitous circumstance that the first approximations were very close to the best estimates that can be made from the data. The reader can verify that, had less good first approximations $m_1=1.31$, $s_1=0.19$ been taken, the corrections would have been $\delta m=-0.0096$, $\delta s=+0.0124$, leading to $m=1.300\pm0.041$, essentially the same result as before.

If the correction calculated for m or s is large, *e.g.*, exceeding the standard deviation of m, as may occasionally happen in experiments in which a considerable proportion of the animals die without tumors, and in which therefore the graphical estimates m_1 and s_1 are rather rough, it may be desirable to proceed to a further approximation. This is done by repeating the calculation, using for m_1 and s_1 not the graphical first approximations, but the corrected values resulting from the first calculation.

The method of calculating the corrections δm and δs breaks down if first approximations m_1 and s_1 absurdly far from the best values are used.

The calculation of the mean log induction time will be particularly of use in experiments of which the object is to decide whether a significant difference is made by some difference in treatment. Andervont and Shear, in addition to the experiment treated in Table III above (in which the pellet containing methylcholanthrene was left in place for 6 weeks), also made an experiment in which the pellet was left in place for 8 to 12 weeks. In this last experiment all the mice got tumors, so the mean log induction time and its standard error can be directly calculated as described previously. The result of the comparison of the two experiments is:

Pellet present for 6 weeks,	1.299 ± 0.042
Pellet present 8 to 12 weeks,	1.185 ± 0.028
Difference	0.114 ± 0.050

The difference, being 2.3 times its standard error, is fairly certainly real (P less than 0.05).

SUMMARY

It is proposed that the mean of the logarithms of the induction times should be used as a means of summarizing the results of experiments in which a carcinogen is applied to a batch of animals and some or all acquire tumors. The method of working up the results of an experiment in which some of the animals fail to develop tumors is described. A table is provided to facilitate the numerical work, and an example is worked out in detail.

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REFERENCES

1. Andervont, H. B., and Shear, M. J. Production of Tumors in Mice Following the Removal of Methylcholanthrene-Cholesterol Pellets. J. Nat. Cancer Inst., **2**:333-334. 1942.
2. Beck, S., and Peacock, P. R. The Latent Carcinogenic Action of 3:4-Benzpyrene; Results of Intermittent Application to the Skin of Mice. Brit. J. Exper. Path., **21**: 227-230. 1940.
3. Bliss, C. I. The Calculation of the Dosage-Mortality Curve. Ann. Appl. Biol., **22**:134-167. 1935.
4. Bliss, C. I. The Calculation of the Time-Mortality Curve. Ann. Appl. Biol., **24**:815-852. 1937.
5. Blum, H. F. Relationships between Spontaneous Tumors of the Lung and Cutaneous Tumors Induced with Ultraviolet Radiation in Strain A Mice. J. Nat. Cancer Inst., **5**:89-97. 1944.
6. Blum, H. F., Grady, H. G., and Kirby-Smith, J. S. Limits of Accuracy in Experimental Carcinogenesis as Exemplified by Tumor Induction with Ultraviolet Radiation. J. Nat. Cancer Inst., **3**:83-89. 1942.
7. Bonser, G. M. The Hereditary Factor in Induced Skin Tumours in Mice: Establishment of a Strain Specially Sensitive to Carcinogenic Agents Applied to the Skin. J. Path & Bact., **46**:581-602. 1938.
8. Bryan, W. R., and Shimkin, M. B. Quantitative Analysis of Dose-Response Data Obtained with Carcinogenic Hydrocarbons. J. Nat. Cancer Inst., **1**:807-833. 1941.
9. Bryan, W. R., and Shimkin, M. B. Quantitative Analysis of Dose-Response Data Obtained with Three Carcino-

genic Hydrocarbons in Strain C3H Male Mice. J. Nat. Cancer Inst., **3**:503-531. 1943.

10. Finney, D. J. On the Distribution of a Variate Whose Logarithm is Normally Distributed. J. Roy. Stat. Soc., Supp., **7**:155-161. 1941.
11. Fisher, R. A. On the Mathematical Foundations of Theoretical Statistics. Phil. Trans. Roy. Soc. London, s.A, **222**:309-368. 1922.
12. Fisher, R. A. Statistical Theory of Estimation. Calcutta: University Press. 1938.
13. Fisher, R. A., and Yates, F. Statistical Tables for Biological, Agricultural and Medical Research. London: Oliver and Boyd. 1938.
14. Iball, J. The Relative Potency of Carcinogenic Compounds. Am. J. Cancer, **35**:188-190. 1939.
15. Shimkin, M. B., and Andervont, H. B. Factors Influencing Carcinogenesis with Methylcholanthrene. III. The Effect of Solvents. U. S. Pub. Health Rep., **55**:537-545. 1940.
16. Shimkin, M. B., and Andervont, H. B. Comparative Carcinogenicity of Three Carcinogenic Hydrocarbons. J. Nat. Cancer Inst., **1**:57-62. 1940.
17. Stevens, W. L. The Truncated Normal Distribution. Ann. Appl. Biol., **24**:847-850. 1937. (Appendix to Bliss, 1937).
18. Stowell, R. E., and Cramer, W. The Effects of Solvents in Methylcholanthrene Epidermal Carcinogenesis. A Comparison of Benzene and Acetone. Cancer Research, **2**:193-197. 1942.

Certain Effects of Egg White and Biotin on the Carcinogenicity of *p*-Dimethylaminoazobenzene in Rats Fed a Sub-Protective Level of Riboflavin*

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Considerable interest in the possible cocarcinogenic action of biotin and in the anticarcinogenic influence of egg white and avidin has arisen since the report by du Vigneaud and his group (2) on the effect of these substances upon the rate of hepatoma formation in rats fed *p*-dimethylaminoazobenzene. These investigators found that rats fed the dye in protective diets containing high amounts of riboflavin developed hepatomas when sufficient biotin was given, and that the effect of biotin was neutralized when the diet contained enough egg white to bind the dietary biotin in the biotin-avidin complex. The livers of the protected rats were found to contain on the average only one-third as much biotin as the nontumor parts of the livers in the animals given biotin (1). Chiefly as a result of these experiments several attempts have been made to retard the growth of various types of neoplasia in human patients and laboratory animals by the administration of egg white or avidin (3, 4, 8, 10). None, however, have been successful.

Smith, Lillie, and Stohlman (9) studied the liver damage in rats fed a high level, 0.1 per cent, of *p*-dimethylaminoazobenzene in partially purified diets containing large amounts of various protein sources, and found that 32 per cent of dried egg white prevented liver tumors and greatly reduced the degree of cirrhosis, while 37 per cent of casein gave slight protection and 53 per cent of yeast or 32 per cent of gelatin none at all. These diets contained at least 5 per cent of yeast, and no symptoms of biotin deficiency in the rats fed egg white were mentioned. The suggestion was made that egg white can partially detoxify the azo dye, and that its protective effect may not be due solely to its avidin content.

The present study deals with the effect of egg white on the incidence of hepatomas in rats fed *p*-dimethylaminoazobenzene in a highly purified diet containing a sub-protective level of riboflavin. Attempts to alter the effect of the egg white by the injection of biotin or by denaturing the avidin in the egg white by heat are reported.

METHODS

Young adult male albino rats of the Sprague-Dawley strain and 120 to 200 gm. in weight were fed the rations *ad libitum*. They were kept in groups of 7 or 8 in screen-bottomed cages. The dye was fed for 4 months and the livers were examined by laparotomy at the end of this period; the animals were then continued on the same basal diets without the dye for another 2 months. At 6 months a final examination of the livers was made.

The basal diets contained either vitamin-free casein (5), 120 gm., or a similar quantity of domestic dried egg white, unheated or heated; glucose monohydrate, 790 gm.; salts, 40 gm.; corn oil, 50 gm.; thiamine chloride, 3 mgm.; pyridoxine hydrochloride, 2.5 mgm.; calcium pantothenate, 7.0 mgm.; and choline chloride, 30.0 mgm. per kgm. Two milligrams of riboflavin per kgm. was added to the diets containing casein. In the other diets the egg white, heated and unheated, was assayed for riboflavin (fluorometric procedure [1]) and only enough of this vitamin was added to bring the total to 2 mgm. per kgm. of diet. The unheated egg white samples assayed 3.0 and 17.0 μgm. of riboflavin per gm., while the latter sample after heating contained 12 μgm. per gm. Each rat received 1 drop of halibut liver oil per month throughout the experiment. The carcinogen, *p*-dimethylaminoazobenzene, was fed at a level of 0.06 per cent, and was incorporated in the diets by dissolving it with heat in the corn oil.

The heated egg white was prepared by autoclaving 150 gm. of the dried domestic product with 750 cc. of water in a shallow pan for 90 minutes at 15 lbs. pressure. It was then dried and ground. No assays

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[1] These assays were kindly made by Mr. B. S. Schweigert.

for avidin were made. Since the animals on the heated egg-white diet continued to lose weight over the first 2 months of the experiment, each egg-white diet in this series was supplemented with 2 per cent of purified casein at the expense of glucose for the following 4 months. Each rat of one group fed the unheated egg white received 2 μgm. of crystalline biotin in 0.2 cc. of saline solution by subcutaneous injection 3 times a week.

RESULTS

The results obtained are summarized in Table I. The first series comprised two groups of rats, one fed the diet containing casein (Group 1) and the other given egg white instead (Group 2). The controls (Group 1) responded to the carcinogen in the same manner as observed repeatedly in previous experiments (5, 6, 7). Although the survival at 4 months was slightly lower than usual, the tumor incidence of 82 per cent at 6 months, and the moderate to severe cirrhosis that developed, were typical for this diet. The substitution of egg white for casein (Group 2) completely prevented the formation of tumors by 6 months, and there was no gross cirrhosis in this group at 4 months. Although both groups consumed on the average the same amount of food and carcinogen, the control group gained weight whereas the egg-white group lost some weight, particularly in the last 2 months. After 3 months the first symptoms of biotin deficiency were noted in the rats fed the egg-white diet. The syndrome, which increased in severity throughout the remainder of the experiment, consisted of a generalized loss of hair, erythematous dermatitis, hunched backs, hypersensitiveness, and depositions of red-brown pigment about the eyes, nose, and paws.

The second series of experiments included 2 groups fed casein and unheated egg white as in the first experiment. In addition one group was fed unheated egg white and given repeated injections of crystalline biotin, while another received heated egg white. The rats in each of these groups ate more food and gained more weight than those in the previous series. Again the rats fed the control diet (Group 3) exhibited a typical incidence of liver tumors, 77 per cent, by 6 months. The rats fed unheated egg white (Group 4) developed a severe biotin deficiency as before, and had a final tumor incidence of only 14 per cent. It is interesting that this group consumed more dye than did the controls.

The injection of 2 μgm. of crystalline biotin 3 times a week into each rat of another group fed unheated egg white (Group 5) completely prevented the biotin deficiency syndrome. The animals ate somewhat more food and gained more weight than did their controls (Group 3). However, only 10 per cent of the rats had hepatomas at 6 months; this incidence is comparable to that of group 4, which did not receive biotin. Similar results were obtained when rats were fed egg white that had been heated to destroy the avidin (Group 6). The animals consumed about the same amount of dye as the controls, and deficiency symptoms were entirely lacking. The incidence of hepatic tumors, 18 per cent at 6 months, was similar to that observed in each of the other groups fed egg white in this series. Apparently the 2 per cent of purified casein that was added to all the egg-white diets of this series after the second month had little or no influence on tumor development.

TABLE I: EFFECT OF EGG WHITE AND BIOTIN ON THE CARCINOGENICITY OF *p*-DIMETHYLAMINOAZOBENZENE IN DIETS CONTAINING A SUB-PROTECTIVE LEVEL OF RIBOFLAVIN

Group	Diet	Av. weight, gm.			Average daily food intake gm./rat	Survival † at 4 mo.	Tumor incidence ‡		Negative survivors at 6 mos.	Cirrhosis at 4 mo.			Biotin deficiency symptoms
		Start	4 mos.	6 mos.			4 mos.	6 mos.		None	Moderate	Severe	
1	Casein	191	193	221	8.0	11/15	4/11	9/11	2	1	8	2	None
2	Egg white	180	185	170	8.0	13/15	0/13	0/13	8	13	0	0	Severe
3	Casein	177	200	220	10.0	13/15	1/13	10/13	0	0	9	4	None
4	Egg white *	182	244	246	11.3	14/15	0/14	2/14	11	12	2	0	Severe
5	Egg white * + injected biotin	175	246	278	10.8	10/15	0/10	1/10	5	5	5	0	None
6	Heated egg white *	181	230	258	10.2	11/15	0/11	2/11	6	8	2	1	None

* 2 per cent purified casein added at the expense of glucose after the second month (see text).
† Survival = Number at 4 months over number at start.
‡ Tumor incidence = Number with tumors over survival at 4 months.

DISCUSSION

The present experiments demonstrate that the replacement of 12 per cent of casein by egg white in a highly carcinogenic purified diet results in a striking decrease in the rate of hepatoma formation. The egg white exerted its effect equally well whether it was fed as the relatively native protein, or after having been heated sufficiently to destroy the avidin present, or when it was supplemented with crystalline biotin.

In the latter 2 instances sufficient biotin was supplied in the diet (heated egg white contains available biotin) and by intestinal synthesis or through deliberate injection to prevent occurrence of the characteristic deficiency syndrome. It is not known whether still higher levels of biotin would have overcome the protective effect of the egg white, but since no increased tumor incidence was observed when biotin was injected, and particularly when heated egg white was fed, the concept of an altered biotin balance (1) does not appear to explain these results. Instead, the anticarcinogenic action of egg white in these experiments seems to be independent of its avidin content.

The original experiments demonstrating the cocarcinogenic effect of biotin on the induction of hepatomas by *p*-dimethylaminoazobenzene were conducted with diets containing casein and/or egg white and high levels, 10 and 20 μgm. per gm. of diet, of riboflavin (2). These diets largely prevented the formation of liver tumors by 150 to 230 days when no excess biotin was given. The tumor incidence was increased to over 50 per cent upon the addition of crystalline biotin or a biotin ester concentrate to the diets at levels of 0.3 to 2.0 μgm. per rat per day. This effect was independent of dietary egg white as long as an excess of biotin was present in the diet. If sufficient egg white was fed to bind all the biotin very few tumors were evident at the end of the experiment.

The diets discussed in the present paper contained a level of riboflavin, 2 μgm. per gm. of diet, which, when casein is fed, is not sufficient to protect against tumor development by *p*-dimethylaminoazobenzene. Yet egg white, heated or unheated, and egg white with injected biotin protected strongly against the effects of the azo dye. It is possible, of course, that the level of riboflavin in the diet may determine the cocarcinogenic action of biotin on the development of liver tumors due to *p*-dimethylaminoazobenzene. Other compounds, possibly present in natural materials such as egg white and casein, or formed via intestinal synthesis, may likewise affect the cocarcinogenic effect of biotin. Thus, for example, Burk and his associates (1) found that 0.05 per cent of *p*-aminobenzoic acid in the diet overcame the action of biotin and prevented the formation of hepatomas by the azo dye.

SUMMARY

Six groups of 15 rats each were fed 0.06 per cent of *p*-dimethylaminoazobenzene in highly purified diets containing a sub-protective level of riboflavin, *e.g.*, 2 μgm./gm. diet, and 12 per cent of protein as vitamin-free casein, unheated dried egg white, or heated dried egg white. Each rat of one group fed unheated egg white also received 2 μgm. of crystalline biotin by subcutaneous injection 3 times a week. The dye was fed for 4 months and followed by the dye-free basal diets for another 2 months. The livers were inspected by laparotomy at 4 months and a final tumor count was made at 6 months.

The incidence of liver tumors at 6 months on the casein diets was 77 and 82 per cent, while on the egg-white diets it varied from zero to 18 per cent. Thus neither the injection of biotin nor the heat denaturation of the avidin present in the egg white destroyed the protective effect exerted by this protein. Symptoms of biotin deficiency developed after 3 months on the unheated dried egg-white diets, but did not appear when biotin was given or if the egg white was heated. The protection against hepatoma formation offered by egg white in these experiments appeared to be independent of any obvious biotin-egg-white relationship. It is important to note that these experiments were performed with diets containing a sub-protective level of riboflavin; the previous reports on the cocarcinogenic effect of biotin concerned diets containing highly protective levels of this vitamin.

REFERENCES

1. Burk, D., Spangler, Juliet M., du Vigneaud, V., Kensler, C., Sugiura, K., and Rhoads, C. P. Biotin-Avidin Balance in *p*-Dimethylaminoazobenzene Tumor Formation. Proceedings, 35th Annual Meeting, American Association for Cancer Research, Inc. Cancer Research, **3**:130-131. 1943.
2. du Vigneaud, V., Spangler, Juliet M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C. P. The Procarcinogenic Effect of Biotin in Butter Yellow Tumor Formation. Science, **95**:174-176. 1942.
3. Kaplan, I. I. One Year Observations of the Treatment of Cancer with Avidin (Egg White). Am. J. M. Sc., **207**: 733-743. 1944.
4. Kensler, C. J., Wadsworth, C., Sugiura, K., Rhoads, C. P., Dittmer, K., and du Vigneaud, V. The Influence of Egg White and Avidin Feeding on Tumor Growth. Cancer Research, **3**:823-824. 1943.
5. Miller, J. A., Kline, B. E., Rusch, H. P., and Baumann, C. A. The Carcinogenicity of *p*-Dimethylaminoazobenzene in Diets Containing Hydrogenated Coconut Oil. Cancer Research, **4**:153-158. 1944.
6. Miller, J. A., Kline, B. E., Rusch, H. P., and Baumann, C. A. The Effect of Certain Lipids on the Carcinogenicity of *p*-Dimethylaminoazobenzene. Cancer Research, **4**:756-761. 1944.
7. Miner, D. L., Miller, J. A., Baumann, C. A., and Rusch, H. P. The Effect of Pyridoxin and Other B Vitamins on the Production of Liver Cancer with *p*-Dimethylaminoazobenzene. Cancer Research, **3**:296-302. 1943.
8. Rhoads, C. P., and Abels, J. C. The Administration of Egg White and Avidin Concentrates to Patients with Cancer. J. A. M. A., **121**:1261-1263. 1943.
9. Smith, M. I., Lillie, R. D., and Stohlman, E. F. The Toxicity and Histopathology of Some Azo Compounds as Influenced by Dietary Protein. Pub. Health Rep., **58**:304-317. 1943.
10. West, P. M., and Woglom, W. H. Abnormalities in the Distribution of Biotin in Certain Tumors and Embryo Tissues. Cancer Research, **2**:324-331. 1942.

Spontaneous Malignant Mixed Tumors of the Rat, and the Successful Transplantation and Separation of both Components from a Mammary Tumor

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Malignant mixed tumors in man are relatively rare, but their occurrence has occasioned considerable discussion of their probable histogenesis. In 1937, Raso (16) reported an osteoid chondrosarcoma of the breast and collected from the literature, dating from 1835, 74 mammary tumors containing bone and cartilage, but only 5 were considered to be true mixed tumors. Saphir and Vass (17), in 1938, reviewed 153 cases of "carcinosarcoma," excluding any tumors containing large amounts of cartilage and bone that might be interpreted as teratomas. They concluded from the morphology of these neoplasms that in all but 3 or 4 cases a dual nature was very questionable. The majority they interpreted as primary carcinomas, evaluating the sarcomatous portion as composed of carcinoma cells showing transitional features, or marked anaplasia, or as a lymphocytic reaction due to chronic inflammation. Other cases they believed to be sarcomas invading metaplastic epithelial structures.

Budd and Breslin (2) reported a malignant mixed tumor of the chest wall, which Ewing considered a primary mammary carcinoma with bone deposits added by a curious type of metaplasia. Mallory diagnosed it as a carcinoma and an osteogenic sarcoma, one invading the other, and Masson believed in this case that he could follow a direct transformation of the epithelioma into osteosarcoma.

Allen (1) described 4 mixed tumors of the mammary glands of dogs, and 1 of the human breast. In 3 of the canine tumors he believed the cartilage was derived directly from adult epithelium. This view was endorsed by Ewing (9). In contrast, Harvey, Dawson, and Innes (12) and Sheldon (18) believed the cartilage and bone of mixed tumors of the salivary glands arise by metaplasia from the connective tissue stroma.

In the mouse, Slye, Holmes, and Wells (19) reported 12 malignant neoplasms of the thyroid gland of which 4 were of dual origin, 3 being carcinosarcomas and 1 a mixed tumor. Loeb (14) reported the transplantation of an adenocarcinoma of the submaxillary gland of a mouse, which developed into mixed tumors and spindle cell sarcomas in the first transplanted generation. A transplantable mixed tumor of the fowl was reported by Foulds (10). The primary growth, which appeared to be a carcinoma of the oviduct, was transplanted for 12 generations with varying success into the pectoral muscles of fowls of the same and different breeds. Bone and cartilage were found in growths of the first and succeeding generations of transplantation. No metastases were recorded, and Foulds concluded that only the epithelial component survived from generation to generation, the stroma being formed anew by the host's connective tissue cells under the influence of the neoplastic epithelial parenchyma.

The frequency with which sarcomatous transformation of the stroma of transplanted mammary carcinomas has been observed by Ludford and Barlow (15) in highly inbred stocks of mice and in studies of these growths *in vitro* has been attributed by these authors to either of two factors; the considerable stimulating action of the carcinoma cells on the fibroblasts, or persistence of the stromal cells through repeated transplantations due to homozygosity of the cells of the graft and of the new host. In each case there was a progressive increase in quantity and cellularity of the stroma, which preceded the appearance of the sarcoma as had earlier been observed by numerous investigators including Ehrlich and Apolant (8), Haaland (11), and Lewin (13); by the latter also in the case of a transplanted adenocarcinoma in a rat. In most cases the authors excluded the probability that these growths could have been mixed tumors from their inception, since they had been under observation for from 5 to as many as 67 generations before the sarcomatous element was identified. In one case (8) the appearance of a mixed tumor persisted for 20 generations, but more commonly the sarcomatous portion overgrew

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the carcinomatous within a generation or two after its recognition and ultimately supplanted it entirely. Ehrlich (7) and Haaland (11) each hastened this separation process by experimental measures. The former used heat or cold to destroy or selectively weaken the carcinomatous portion, and the latter inoculated the mixed tumor into mice experimentally immunized to mouse carcinoma cells; in both instances some pure sarcomas resulted, whereas the control series continued as mixed tumors.

Mixed fibroepithelial neoplasms occur spontaneously (4), and can be readily induced with comparative frequency in the rat; often both components appear to be malignant. In 1928 Bullock and Curtis (3) reported a *Cysticercus* tumor of the rat's liver classified as a carcino-osteochondrosarcoma. Peritoneal metastases were abundant, and microscopic examination revealed scattered islands of osteoid tissue and

Spontaneous Mixed Tumors of Rats

A study of the spontaneous mixed tumors that occurred in the rats of our colony offers pertinent data regarding the nature of these growths. Metastases frequently occurred and were often a direct factor in the death of the hosts; examination of these metastatic growths established beyond a doubt the malignant character of one or more of the tissues observed in the primary tumor.

Among the spontaneous neoplasms 27 mixed tumors of the mammary gland, uterus, and face were observed (Table I). In the mammary gland 41 malignant growths were found; 24 were mesodermal, 12 epithelial, and 5 were mixed tumors. The sarcomatous portions were mixed with portions of adenocarcinoma in 3 instances, while 2 tumors had these elements plus portions of squamous cell carcinoma. Only one of

Table I: The Number and Percentage of Spontaneous Tumors at 3 Sites That Were of Each Morphological Pattern

		Classification				Mixed tumors			
Location		Fibrosarcoma	Adenocarcinoma	Sq. cell carcinoma	Adenoacanthoma	Sarcoma and adenocarcinoma	Sarcoma and sq. cell carcinoma	Sarcoma and adenocarcinoma and sq. cell carcinoma	Total
Breast	No.	24	9	1	2	3	0	2	41
	%	59	22	2	5	7		5	100
Uterus	No.	68	12	24	2	3	8	1	118
	%	58	10	20	2	2	7	1	100
Face	No.	21	0	57	1	0	10	0	89
	%	24		64	1		11		100
Total		113	21	82	5	6	18	3	248

cartilage in the diaphragm and neoplastic epithelial cells within the lumina of the vessels in the uninvolved part of the cyst wall. This tumor was one of 3, in a series of 2,100 induced mesothelial tumors, that contained neoplastic epithelium. In 1936 Dunning, Curtis, and Bullock (5) reported a total of 9 mixed tumors in a series of about 900 benzpyrene and dibenzanthracene neoplasms of the rat. In this article were included 25 mixed tumors of the mouse in a series of some 500 benzpyrene tumors. Later, in 1940 (6), 17 mixed tumors were discovered among approximately 900 subcutaneous methylcholanthrene neoplasms. The epithelial component of the tumors previously mentioned was of mammary origin and probably resulted from direct local action of the carcinogen on the glandular tissue or the areas of metaplastic squamous epithelium frequently observed in the mammary ducts adjacent to foci of injected carcinogen. Metastases were scanty and contribute little evidence for the malignancy of either component, since the hosts were sacrificed soon after the tumors were observed.

these metastasized, with the production of extensive areas of adenocarcinoma in the lungs. One of the 2 that contained both adenocarcinoma and squamous cell carcinoma (R2572) was used in the transplantation studies described here.

The uterus was the site of the largest number of mixed tumors in these rats. Of a total of 118 malignant neoplasms in this organ, 68 were sarcomas, 38 were carcinomas, and 12 were mixed tumors. Most of the latter were composed of squamous cell carcinoma mixed with mesodermal elements. Metastases were found in the peritoneum and abdominal viscera in 6 cases, with both components of the tumors present in 5.

Another frequent site of malignant mixed tumors in the rat is the skin of the face near the orbit. Here 89 malignant neoplasms were found; 57 were squamous cell carcinoma, 1 contained squamous and glandular epithelium, 21 were fibrosarcomas, and 10 were mixed tumors made up of sarcoma and squamous cell carcinoma. Metastases were found from only 3 of these

10 mixed tumors. In 1 case the secondary growths were composed of pure squamous cell carcinoma, in another of pure fibrosarcoma, and in the third both components were present.

From examination of these data it seems probable that in the rat malignant mixed neoplasms composed of 2 independent neoplastic components growing as a single tumor occur at sites where growths composed of each component are frequent. Either or both of the components may metastasize. In all, 27 mixed tumors have been observed, with metastases occurring in 10, of which 6 showed both malignant mesodermal and malignant epithelial components. In order to evaluate further the nature of a mixed tumor the following transplantation experiments were undertaken.

EXPERIMENTAL PROCEDURE

Transplantation History of Mixed Tumor R2572

The tumor selected for transplantation studies was R2572. This was a soft, mottled, encapsulated mass in the subcutaneous tissues of the left groin. It had grown rapidly, was free from the skin and underlying muscle, and measured $4.5 \times 4.0 \times 2.2$ cm. when excised for transplantation. A diagnosis of papillary cyst adenocarcinoma was made from a frozen section, which was later confirmed from a paraffin section (Fig. 1).

The bearer was an 849 day old A×C female from the 20th brother-by-sister generation of Line 9935. She died 65 days after excision of the primary growth with no recurrence, but with an early squamous cell cancer of the lower jaw.

Fragments of the primary mammary tumor were implanted in the subcutaneous tissues of both sides of each of 6 males of the same inbred line as the bearer of the primary growth. The grafts all grew progressively, and microscopic examination of each of the resultant neoplasms revealed areas of adenocarcinoma (Fig. 2) not unlike the primary tumor. In addition, 2 tumors showed areas of fibrosarcoma (Fig. 3) with only a few scattered epithelial elements, and 3 contained large areas of squamous cell carcinoma (Fig. 4) and adenoacanthoma (Fig. 5).

The second generation consisted of 8 females similarly implanted with grafts from one of the tumors from the first transplanted generation. All the grafts grew progressively and microscopic study revealed tumors showing all the morphological variations observed in the previous generation and, in addition, 3 growths that contained areas of bone and cartilage (Fig. 6). The presence of bone was detected in the gross at the time of the postmorten examination as well as in the histologic sections.

Mixed tumors continued to appear in the third and fourth transplanted generations, but it was obvious that segregation was taking place. The squamous cell component was not observed after the second generation, and the sarcomatous and bony component was found in only about half of the growths. It seemed feasible, therefore, to attempt to separate the epithelial and connective tissue elements. Table II shows by generation the history and classification of the transplanted fragments.

Transplantation Studies

Procedure.—In preparation for the transplantation of the fifth generation 9 grafts, each weighing about 10 mgm., were cut from one of the mixed tumors (Fig. 7) of the fourth generation. Each graft was cut in half and one half was again divided and implanted on the right and left sides of each of 9 rats. The other half was fixed in Zenker's fluid for serial sectioning.

Animals.—The rats used in the transplantation studies were all of the same inbred strain, A×C Line 9935.

Histologic features of transplanted tumors.—Examination of sections of the 9 tumor fragments revealed 5 to be pure adenocarcinoma; 3 were a mixture of adenocarcinoma and fibrosarcoma, and 1 contained areas of cartilage in addition. The host into which the other half of the tumor fragment containing cartilage had been implanted died after 41 days with 2 tumors, each containing bone and cartilage. The growths were then excised from the left sides of the 8 remaining rats and spaced serial sections were examined. Five appeared to be pure adenocarcinomas, and 3 were mixed tumors corresponding in morphology with the other half of the graft, previously examined.

Results.—On the 89th day after implantation 1 of the 5 rats in which half of the graft and the tumor on the left side had shown only adenocarcinoma was selected and 10 rats were inoculated with fragments from the growth on the right side. A section of the tumor used for inoculation of generation 6A is shown in Fig. 8. For the other series a tumor obviously containing bone was selected from the 3 that had grafts containing fibrosarcoma, and mixed tumors on the left side. Ten females were inoculated with grafts from this tumor (Fig. 9). All the grafts in generation 6B grew progressively and all the resultant tumors were distinctly bony. Seven proved to be mixed tumors and 3 were osteochondrosarcomas. In succeeding generations all the growths have been bony and a decreasing percentage have contained epithelial remnants. Fig. 10 is a representative section of one of these tumors, showing malignant stroma and adult bone. Lung metastases are frequent from growths of this series, and all have been bony in structure. Fig. 11 shows the lungs and pericardium studded with small bony metastases from

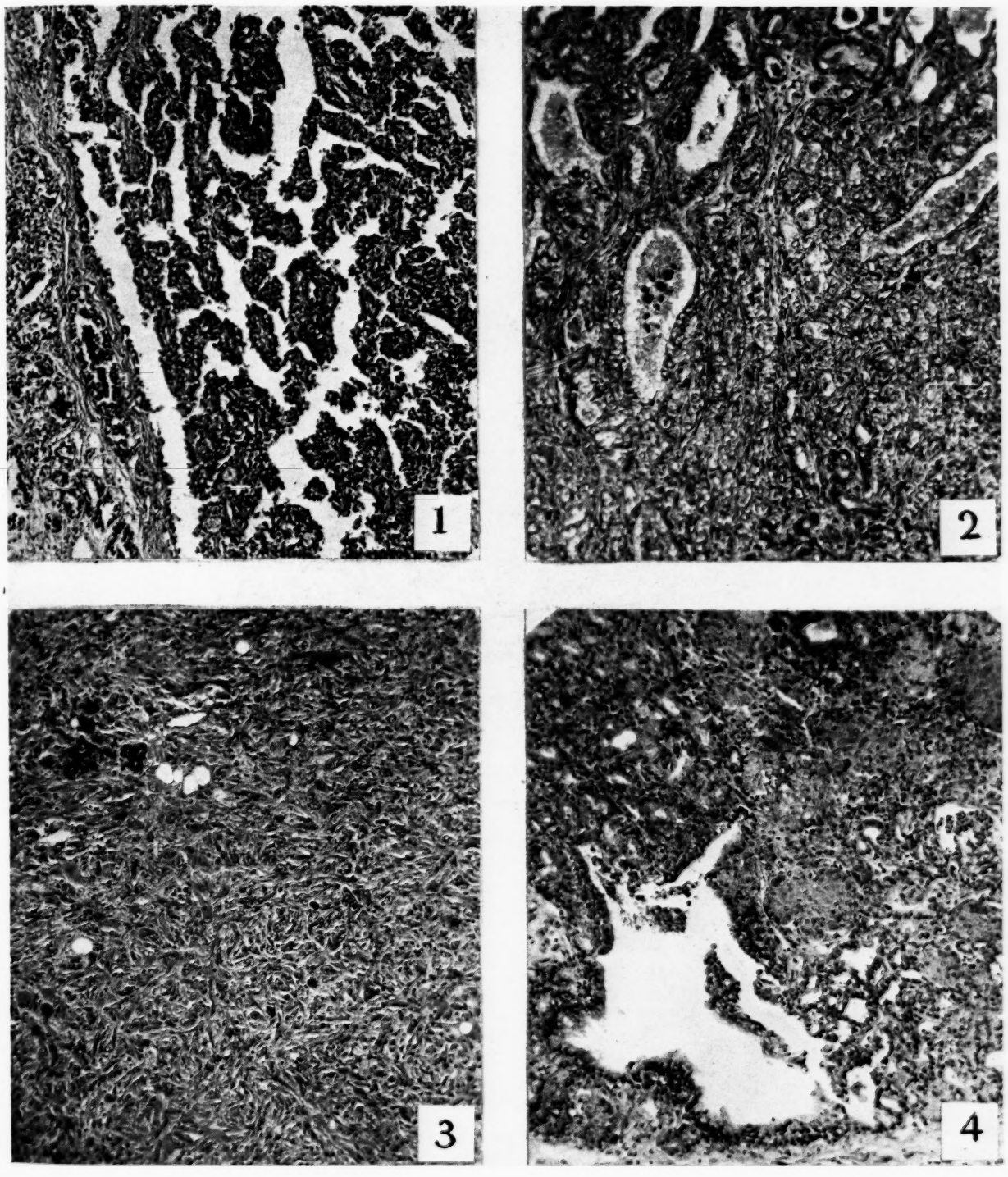
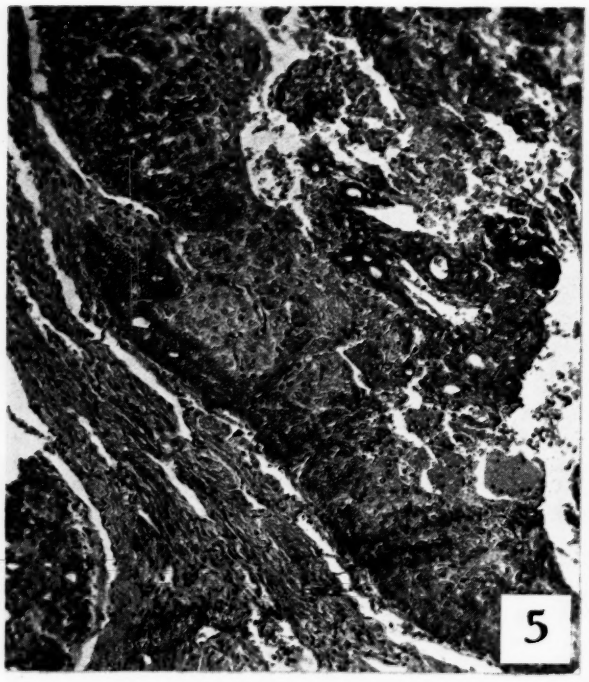
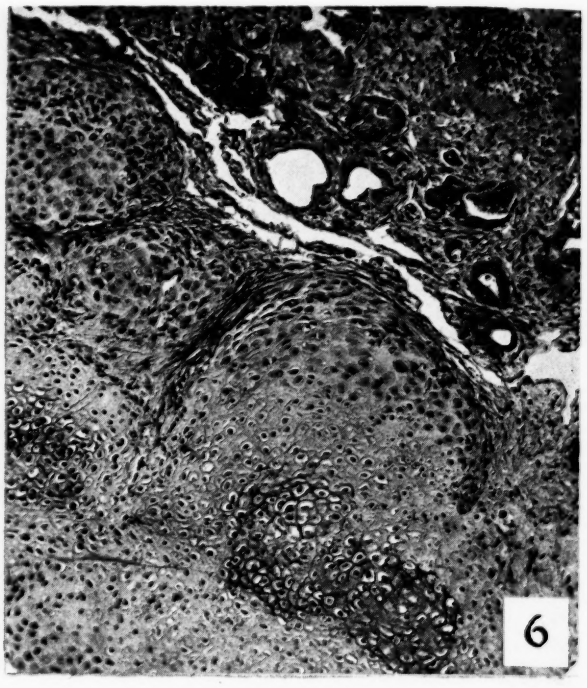
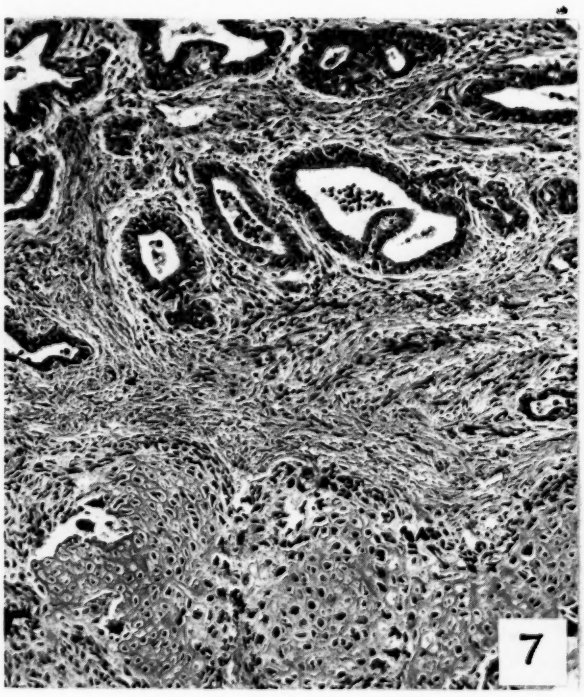


FIG. 1.—Primary spontaneous tumor. R2572. Mag. × 95.
FIG. 2.—Adenocarcinoma in first generation transplant. R2572/1A. Mag. × 95.
FIG. 3.—Fibrosarcoma in first generation transplant. R2572/1A. Mag. × 95.
FIG. 4.—First generation transplant showing a squamous cell carcinoma and adenocarcinoma. R2572/1A. Mag. × 95.







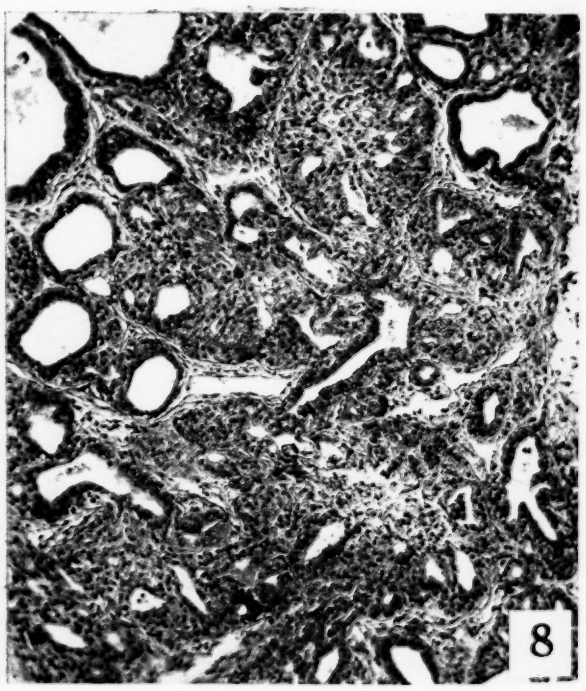


FIG. 5.—Adenoacanthoma in first generation transplant. R2572/1A. Mag. × 95.
FIG. 6.—Second generation transplant showing cartilage and adenocarcinoma. R2572/2A. Mag. × 95.
FIG. 7.—Fourth generation transplant. Mixed tumor showing cartilage, bone, and adenocarcinoma. R2572/4A. Mag. × 95.
FIG. 8.—Adenocarcinoma in fifth generation transplant. R2572/5A to 6A. Mag. × 95.

a tumor of generation 8B, and Fig. 12 is a section through one of these metastatic foci.

The 5 succeeding generations from generation 6A, which was composed solely of adenocarcinoma, have contained no bone or cartilage. In the tenth generation thelium, but this tumor now appears to be a pure adenocarcinoma. Metastases from this component had not been observed until the tenth generation. Fig. 13 shows extensive involvement of the lungs with metastases from one of the tumors in the tenth genera-

TABLE II: TRANSPLANTATION HISTORY BY GENERATION OF R2572

No. of rats	Sex	Gen.	Age inoc., days	Days to death	Tpl. tumor history	Classification				
						Adeno-carcinoma	Sq. cell carcinoma	Fibro-sarcoma	Osteo-chondro-sarcoma	Lung metastases
6	♂	1A	71	227	++	6	3	2	0	0
8	♀	2A	83	148	++	8	4	6	3	0
9	♀	3A	100	147	++	9	0	4	2	0
10	♀	4A	122	132	++	10	0	4	3	0
9	♀	5A	248	106	++	9	0	5	4	1
10	♀	6A	156	69	++	10	0	0	0	0
10	♀	6B	113	96	++	7	0	10	10	1
11	♀	7A	149	67	++	11	1	0	0	0
8	♀	7B	335	125	++	5	0	8	8	2
10	♀	8A	170	75	++	10	0	0	0	0
10	♀	8B	403	97	++	3	0	10	10	4
2	♀	9A	182	56	++	2	0	0	0	0
6	♂	9B	576	72	++	1	0	2	6	2
4	♀	9C	135	52	++	0	0	1	3	0
38	♀	10A	119	107	++	38	0	4?	0	7
8	♂	11A	94	66	++	8	0	0	0	0
5	♀	11A	91	77	++	5	0	0	0	0

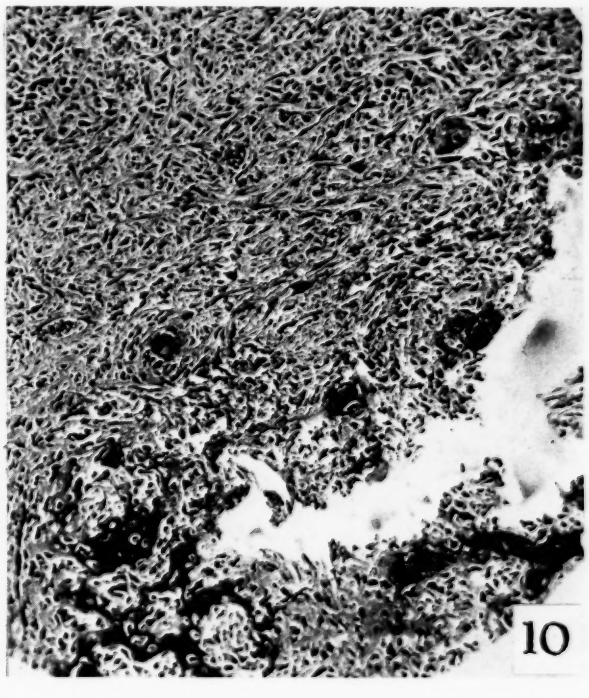
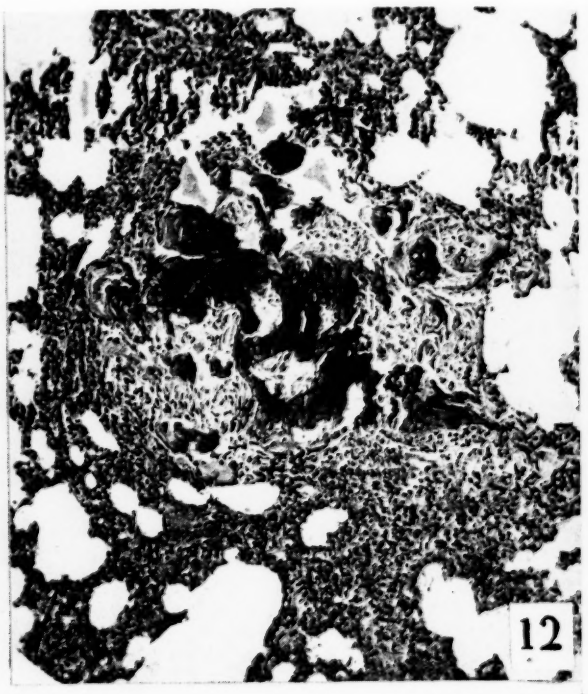


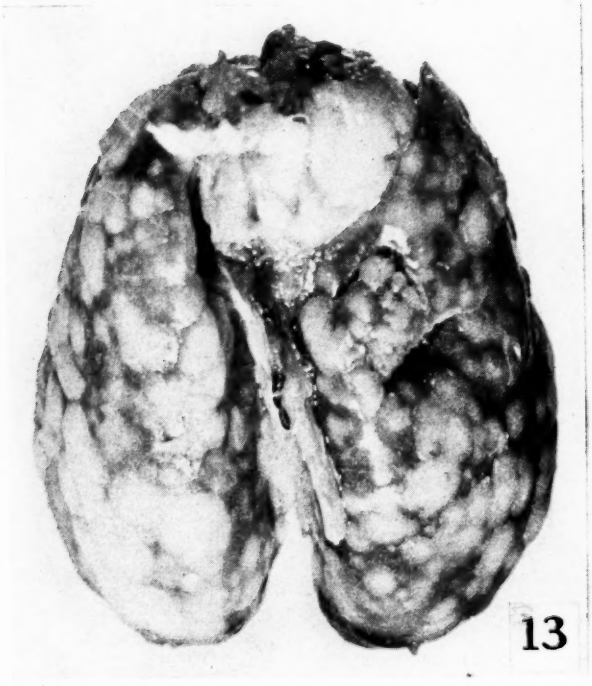
FIG. 9.—Mixed tumor in fourth generation transplant showing bone, cartilage, and adenocarcinoma. R2572/5A to 6B. Mag. × 95.

FIG. 10.—Seventh generation transplant showing adult bone and malignant stroma. R2572/7B. Mag. × 95.

4 of the 38 growths examined showed a somewhat more cellular stroma than is characteristic, and are therefore shown in Table II in the fibrosarcoma column with a question mark. One growth in the seventh generation contained a few islands of squamous epi-tion, and a section (Fig. 14) reveals the epithelial nature of one of these secondary growths.

Remarks.—Spontaneous mixed tumors of rats occurred frequently in the mammary gland, uterus, and face. Metastases from some of these were com-





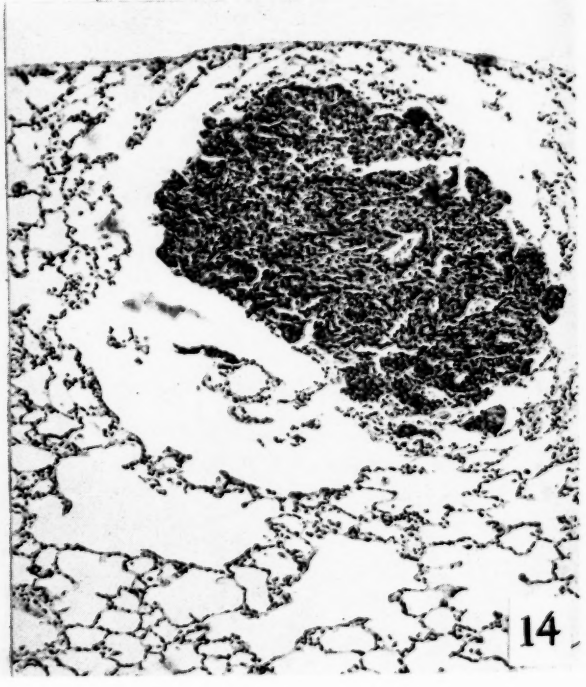


FIG. 11.—Eighth generation transplant showing extensive bony metastases in the lung and pericardium. R2572/8B.
FIG. 12.—Eighth generation transplant showing extensive bony metastasis in the lung. R2572/8B. Mag. × 95.
FIG. 13.—Tenth generation transplant showing extensive lung metastases from the epithelial component.
FIG. 14.—Lung metastasis of the tenth generation transplant. R2572/10A. Mag. × 95.

posed of both epithelial and mesodermal portions, whereas from others only one or the other of these elements was represented. It was possible to separate an epithelial and a mesodermal constituent from a transplanted mixed tumor and to carry each one separately for several successive generations. In one respect this neoplasm was not unlike the fowl tumor reported by Foulds (10) or the mouse tumor by Loeb (14). The few sections of the primary growth that had been preserved and examined microscopically failed to show the dual nature of the neoplasm. The fact that fibrosarcoma was identified in 2 of the 6 rats of the first generation of transplantation and was present in a third, which was used for inoculation and produced 6 mixed tumors out of the 8 in the second generation, pretty well eliminates the probability that the fibrosarcoma was initiated anew in each host by the stimulating action of the neoplastic epithelium. Further, the fact that the adenocarcinomatous portion of the tumor has been carried for 9 successive generations since the separation without exercising this stimulating action on the fibroblasts of the new hosts leaves little doubt that this tumor was composed of malignant epithelium and connective tissue, and that these elements were separable and both autonomous.

SUMMARY

1. Twenty-seven experimentally induced mixed fibroepithelial neoplasms are discussed. Metastases were observed from only 1 of these, and both components were identified.

2. Twenty-seven malignant mixed fibroepithelial spontaneous neoplasms involving the uterus, mammary gland, and face are reported. At these sites both malignant epithelial and connective tissue tumors were frequent. Metastases were examined in 10 of these cases. In 3 the secondary tumors were epithelial, in 1 only the sarcomatous element metastasized, and in 6 cases both components were identified in the secondary growths.

3. Transplantation studies of a spontaneous mammary neoplasm proved it to be a mixed tumor composed of malignant epithelium and mesothelium.

4. After the fifth generation of transplantation the 2 components were separated and are being propagated as a pure adenocarcinoma in one series and an osteochondrosarcoma in the other.

REFERENCES

1. Allen, A. C. So-Called Mixed Tumors of the Mammary Gland of Dog and Man. With Special Reference to the General Problem of Cartilage and Bone Formation. Arch. Path., **29**:589-624. 1940.
2. Budd, J. W., and Breslin, F. J. Carcino-Osteogenic Sarcoma. A Malignant Mixed Tumor of the Chest Wall: Report of a Case. Am. J. Cancer, **31**:207-211. 1937.
3. Bullock, F. D., and Curtis, M. R. A Cysticercus Carcino-osteochondro-Sarcoma of the Rat Liver with Multiple Cysticercus Sarcomata. J. Cancer Research, **12**:326-349. 1928.
4. Bullock, F. D., and Curtis, M. R. Spontaneous Tumors of the Rat. J. Cancer Research, **14**:1-115. 1930.
5. Dunning, W. F., Curtis, M. R., and Bullock, F. D. The Respective Rôles of Heredity and Somatic Mutation in the Origin of Malignancy. Am. J. Cancer, **28**:681-712. 1936.
6. Dunning, W. F., Curtis, M. R., and Eisen, M. J. The Carcinogenic Activity of Methylcholanthrene in Rats. Am. J. Cancer, **40**:85-127. 1940.
7. Ehrlich, P. Experimentelle Studien an Mäusetumoren. Ztschr. f. Krebsforsch., **5**:59-81. 1907.
8. Ehrlich, P., and Apolant, H. Beobachtungen über maligne Mäusetumoren. Berl. klin. Wchschr., **42**:871-874. 1905.
9. Ewing, J. Neoplastic Diseases. A Treatise on Tumors. Fourth edition. Philadelphia & London: W. B. Saunders Company. 1940, p. 148.
10. Foulds, L. A Transplantable Carcinoma of a Domestic Fowl, with a Discussion of the Histogenesis of Mixed Tumours. J. Path. & Bact., **44**:1-18. 1937.
11. Haaland, M. Contributions to the Study of the Development of Sarcoma under Experimental Conditions. Third Scientific Report, Imperial Cancer Research Fund, London. 1908, pp. 175-261.
12. Harvey, W. F., Dawson, E. K., and Innes, J. R. M. Debatable Tumours in Human and Animal Pathology. London, Oliver and Boyd, 1940.
13. Lewin, C. Experimentelle Beiträge zur Morphologie und Biologie bösartiger Geschwülste bei Ratten und Mäusen. Zeitschr. f. Krebsforsch., **6**:267-314. 1908.
14. Loeb, L. Ueber Sarkomentwicklung bei einem drüsenartigen Mäusetumor. Berl. klin. Wchnschr., **43**:798-802. 1906.
15. Ludford, R. J., and Barlow, Hilda. Sarcomatous Transformation of the Stroma of Mammary Carcinomas That Stimulated Fibroblastic Growth *in Vitro*. Cancer Research, **5**:257-264. 1945.
16. Raso, M. I Condroosteomi e i Condroosteosarcomi della Mammella Umana. Pathologica, **29**:229-260. 1937.
17. Saphir, O., and Vass, A. Carcinosarcoma. Am. J. Cancer, **33**:331-361. 1938.
18. Sheldon, W. H. So-Called Mixed Tumors of the Salivary Glands. Arch. Path., **35**:1-20. 1943.
19. Slye, M., Holmes, H. F., and Wells, H. G. The Comparative Pathology of Cancer of the Thyroid, with Report of Primary Spontaneous Tumors of the Thyroid in Mice and in a Rat. J. Cancer Research, **10**:175-193. 1926.

The Development of Highly Malignant Tumor Strains from Naturally Occurring Avian Lymphomatosis

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INTRODUCTION

The economic importance of lymphomatosis is attested by the huge losses that it causes in all sections where poultry are kept. Much work has been done during the last two decades on the transmission and etiology of this disease. One of the greatest handicaps in its experimental study is the apparent long and variable incubation period. Deaths from naturally occurring lymphomatosis have been noted at this laboratory at ages ranging from less than 30 days to as long as birds were kept (over 1,400 days). Numerous investigators have reported the artificial transmission of various forms of the disease. It is generally thought that erythroblastosis and granuloblastosis are easily transmitted (10), but the transmissibility of lymphomatosis remains a moot question. A review of the literature will not be made here since this field has recently been covered by Lee and his group (9), Olson (10), Johnson (6), Feldman and Olson (2), and Jungherr (7).

It has long been recognized that the development of a highly virulent strain of lymphomatosis would be extremely advantageous in the experimental study of the disease. The purpose of this paper is to report experiments on the development of highly virulent tumor strains from cases of naturally occurring lymphomatosis.

MATERIALS AND METHODS

Donors.—The birds that supplied the original material for inoculation were obtained from the control flock of this laboratory. The laboratory flock originated from 9 different strains of pedigreed white leghorn chickens from widely different geographic areas. These birds were uninoculated and none of them had ever been in contact with inoculated stock. They can, therefore, be considered as representative cases of naturally occurring lymphomatosis.

During the past 5 years the naturally occurring disease in the control flock was for the most part neural or visceral lymphomatosis, with a few cases of ocular lymphomatosis. Erythroblastosis, granuloblastosis, myelocytomatosis, and osteopetrosis were extremely rare. Except for lymphomatosis and sporadic cases of coccidiosis, no clinical evidence of disease has been present in this flock since research at the laboratory was initiated in 1939.

All tissues used for inoculation were representative of the various forms of lymphomatosis. Variations in the microscopic picture did occur among the birds studied, but in all cases the diagnosis of lymphomatosis was confirmed. Of the entire series of donors used only 2 birds, E1121A and F268W, showed an abnormal blood picture at the time of necropsy; namely, an increase in the number of erythroblasts, hemocytoblasts, and thrombocytes:

Inoculum.—All prospective donors were killed by electrocution and tissues for inoculation were removed with aseptic precautions. After the removal of a sample for microscopic study the tumor was triturated in a mortar with 4 parts of 0.85 per cent NaCl solution to obtain viable cells for Group I inoculations. For all other inoculations and subsequent passages the tissue was passed through a mincer (11), suspended in 3 to 4 parts of 0.85 per cent NaCl solution, and filtered through a single layer of sterile cheesecloth.

All inoculations were made with a Luer type syringe and needle by routes indicated in Table II. The dosages ranged from 0.1 to 1.0 ml.

Recipients.—All chicks were obtained from pedigreed white leghorn chickens maintained in quarantine and used in a genetic study of resistance and susceptibility to lymphomatosis (17). The chicks used in this study came from matings that had been classified as relatively susceptible to naturally occurring lymphomatosis. With two exceptions (Groups II and III, Table I, raised in an electric canopy brooder) all groups of birds were maintained in wire battery brooders and growing batteries during the course of the transmission experiments. Except for Group III, which was maintained in isolation, the birds within this experiment receiving different inocula were not isolated from each other, but were protected from direct or indirect contact with birds of other experiments.

RESULTS

Original inoculations.—The incidence, survival period, and gross tumor involvement of visceral organs of birds injected at 1 to 2 days of age with tissues of normal and lymphomatous organs are summarized in Table I. Suspensions of the organs were injected by the intraperitoneal route at a dosage of 0.5 ml.

The number that developed tumors during the experimental period of 86 to 120 days varied greatly among the chickens receiving the different inocula. Among the 2 groups inoculated with kidney and liver respectively of E257-0, no birds were positive, whereas all other lymphomatous inocula produced tumors in some of the birds inoculated. A relatively high number of takes was obtained with 4 of the 11 inocula tested; the highest was obtained with the tumorous lung of E809N, which produced 13 takes out of a total of 17 chicks inoculated, with an average survival period of only 11 days. Inocula prepared from the liver and spleen of E1121A also produced many tumors, with average survival periods of 22 days and 30 days respectively. A large number of tumors was obtained from the liver of E1110R_3. However, the average survival period was comparatively long although a number of early cases appeared, making passage at 15 days possible.

Although there was considerable variation in the number of tumors, the lesions produced by the various inocula were very similar when manifestations occurred early. For groups having more than 1 positive bird the organs and tissues showing gross tumor involvement (Table I) are similar for the various inocula used. The average number of organs thus involved ranged from 3.3 to 6.0, but no separation or gradation can be made on the basis of gross pathology or organ specificity.

Typical of the early manifestation is bird F73I_2. Eleven days after intraperitoneal inoculation with a suspension of the lung of E809N, this bird appeared weak and lethargic and showed a definite distention of the abdomen. Upon necropsy a peritonitis, especially in the region of the inoculation site, was observed. The abdominal wall in this area was much thickened, and infiltrated with tumor. The liver, although somewhat enlarged, showed no visible lesions; however, there was a massive involvement of the peritoneal tissues in the hilar region of the liver that extended over the spleen, proventriculus, and part of the pancreas. Involvement of the mesentery, apparently perivascular in nature, followed the course of the larger blood vessels. The pancreas and kidneys were greatly enlarged and tumorous. The testicles, gonad base, and adrenals were enlarged and seemed to be diffusely invaded. Just posterior to the adrenals was a gray tumor that appeared to be an extension of the adrenal involvement.

Birds inoculated intraperitoneally with liver of E1110R_3 reacted somewhat differently in that almost all those that were positive had liver involvement, either with focal areas of gray tumor or a diffuse

TABLE I: RESULTS OF INJECTING NORMAL AND LYMPHOMATOUS MATERIAL INTO THE PERITONEAL CAVITY OF YOUNG CHICKS

Source of inoculum	No. chicks inoculated	No. with tumors	Average survival period,* days	Gross involvement	
				Tissues †	Av. no. per pos. bird
Group I. Lymphomatous					
Nerve, composite	25	3	16	ab,li,sp,pa,in	3.3
Kidney, E257-O	20	0			
Liver, E257-O	19	0			
Lung, E809N	17	13	11	ab,ad,ki,li,sp,pa,pr,pe,me	6.0
Liver, E1121A	19	10	22	ab,li,sp,go,ki,pa,pe,pr,me	4.3
Spleen, E1121A	18	7	30	ab,ad,ki,li,sp,pa,pe	5.1
Liver, E1121M	19	1	9	ab,pa,pe,go	4.0
Group II. Lymphomatous					
Liver, E1110R_3	19	14	56	ab,go,he,ki,li,pa,pe,pr,sp	3.4
Lung, F258E_2	20	1	34	li,he,pa	3.0
Heart, F258E_2	19	3	47	ki,sp,he,ne	1.3
Blood cells, composite	18	3	73	li,ki,sp,he	3.3
Group III. Normal tissue					
Lung, composite	17	0	—		
Liver, "	20	1	—	li,sp	2.0
Kidney, "	14	0			

* Average number of days from inoculation to death of positive birds, except those that were killed.

† ab = abdominal wall, ad = adrenal, go = gonad, he = heart, ki = kidney, li = liver, me = mesentery, ne = nerve, in = intestine, pa = pancreas, pe = peritoneum, pr = proventriculus, sp = spleen.

All birds were killed and examined after an experimental period of 86 days for Group I, and 120 days for Groups II and III.

miliary infiltration. Other organs showed similar involvement but only occasionally were there peritoneal effusion or growths of the serosal membrane.

Birds inoculated with normal lung, liver, or kidney prepared and inoculated in the same manner as the lymphomatous material did not show significant changes. Among 51 thus injected no deaths occurred during the 120 days of the experiment. At the termination of the experiment all birds were killed for necropsy. One was found to have involvement of the liver and spleen. Although the birds in this experiment came from matings that had shown a low incidence of lymphomatosis in the progeny, which were kept under partial quarantine, this one case, occurring at 120 days, was probably due to natural causes.

Serial passage of strains RPL[1]-14, -15, -16, and -17.—The 4 strains to be described were originated and maintained by using the first birds showing typical pathological alterations as donors in the establishment and subsequent passage of the strain. Results of serial passage inoculations with strains RPL-14, -15, -16, and -17 are given in Table II.

Since the early passages are the most important in the establishment of new strains, results for the first 5 are given individually. Data obtained in subsequent passages were similar; hence the mean of passages 6 to 10 and passages 11 to 15, 16, and 19 are given. Birds listed as positive were those that showed a tumor at the site of inoculation. The index used for visceral involvement was the average number of viscera or tissues showing macroscopic tumors. This figure may be taken as an index of metastases in cases of intramuscular or subcutaneous inoculations. Many birds, especially in the early passages, were killed to serve as donors. With two exceptions, all donors used had metastases to the viscera, but the extent was usually less than in those that died; hence they were omitted in the calculation of visceral involvement at death. The percentage in the column headed "death rate" gives the proportion that died with tumorous organs within 30 days after inoculation, and since birds that were killed may have lived for more than 30 days they are omitted from that analysis. The survival period is here defined as the number of days from inoculation to death of those that are positive. The passage interval is the number of days from the inoculation of the donor to inoculation of recipients for the next passage.

Strain RPL-14.—Lymphomatous lung tissue of E809N produced diffuse tumors of the abdominal wall and visceral organs and tissues so rapidly in 2 day old chicks that passage was carried out 10 to 15 days after inoculation. No tumors resulted from intramuscular injections of the same inoculum into chicks differing only in that they were 14 days older than those injected intraperitoneally. In the second passage, however, intramuscular takes were obtained in 2 out of 10 chicks that were 17 days old at the time of inoculation, as compared with 15 out of 29 positives by the intraperitoneal route. A rapid increase in the incidence of takes by intramuscular inoculations took place after the fourth passage; thus, out of 100 inoculated, 98 were positive. A limited intraperitoneal test at the fifth passage indicated that there was no significant change in effectiveness during the intervening 2 intramuscular passages. Inoculations made in the second and third passages by the subcutaneous route also induced local tumors. Visceral involvement was extensive in positive birds of the original inoculation; in the next 6 passages it was rather limited, but beginning with the eighth visceral metastasis started to increase so that an average of 5 organs showed gross involvement in the 14th passage. The changes in the death rate coincided closely with the changes in the extent of metastasis. The average survival time also increased during these passages. The age of the birds may have had some influence on the changes mentioned above, especially the survival time, since somewhat older birds were used in the later passages.

Strain RPL-15.—A suspension of the lymphomatous spleen of E1121A injected intraperitoneally produced tumors of the abdomenal wall and the viscera in 7 of 18 two-day old chicks inoculated. The same material intramuscularly gave negative results in 17-day old chicks.

The first passage with this strain was made at 15 days, and after its third passage the interval was reduced to 8 to 12 days. The percentage of positive birds obtained by intraperitoneal and intramuscular inoculations increased during the first 5 passages. The change was most distinct by the latter route, the incidence being zero in the first passage, 1 positive out of 10 in the second, and 17 out of 20 in the third passage. For the sixth to 16th passages, only the intramuscular route was used, with an incidence of 94 positive cases out of 95 birds inoculated. The pattern of metastasis, death rate, and survival period during the several passages was similar to that obtained with RPL-14 although a somewhat higher malignancy is indicated.

Strain RPL-16.—Since the lymphomatous liver that gave rise to RPL-16 came from the same bird that supplied the spleen for RPL-15, they may be considered the same strain. However, inasmuch as the 2 were handled entirely separately after the excision of the tumors from the donor and for convenience in

[1] An abbreviation of Regional Poultry Research Laboratory of the Agricultural Research Administration, United States Department of Agriculture, East Lansing, Michigan.

TABLE II: THE SERIAL PASSAGE OF STRAINS RPL-14, -15, -16, AND -17

Passage no.	Location of tumor used as inoculum	Route	Age of chicks at inoc., days	No. inoc.	No. pos.	Visceral involvement	Death rate, %	Survival, days	Passage interval, days
				RPL-14					
1	Lung	i.p.	2	17	13	5.1	79	11	10,15
		i.m.	16	10	0	—	0	—	—
2	Abdominal	i.p.	3,11	29	15	1.0	8	35	12
	wall, pancreas,	sq.	17	10	5	—	0	—	—
	gonad	i.m.	17	10	2	—	0	—	—
3	Serosa	i.p.	1	6	5	2.0	75	13	—
		sq.	1	7	1	—	0	—	—
		i.m.	1	18	11	0.1	47	11	9
4	Muscle	i.m.	10	10	9	1.0	50	11	7
5	Muscle	i.m.	3	11	11	1.0	20	15	8
		i.p.	3	4	3	1.6	75	14	
6-10	Muscle	i.m.	11-17	49	48	3.2	32	21	11-12
11-15	Muscle	i.m.	17-25	36	36	4.5	85	24	10-12
				RPL-15					
1	Spleen	i.p.	2	18	7	4.2	33	30	15
		i.m.	17	9	0	—	0	—	—
2	Abdominal								
	wall, ovary,	i.p.	3	17	6	3.0	8	23	12
	liver,	i.m.	17	10	1	—	0	—	—
	pancreas								
3	Serosa	i.p.	1	17	15	2.5	83	14	9,14
		i.m.	1	20	17	1.0	69	13	7,14
4	Serosa	i.p.	1,10	24	16	1.9	55	11	—
	Muscle	i.m.	1,8	29	29	2.4	100	13	9,11
5	Muscle	i.p.	3	4	3	3.5	50	14	—
	"	i.m.	3	12	12	1.8	66	14	8
	Liver	i.p.	5	16	16	3.9	100	14	—
	"	i.m.	5	9	9	4.9	100	16	—
6-10	Muscle	i.m.	11-16	50	49	4.4	43	20	10-12
11-16	Muscle	i.m.	14-28	45	45	4.0	72	19	9-10
				RPL-16					
1	Liver	i.p.	2	19	10	3.6	35	22	27,36
		i.m.	17	10	0	—	0	—	—
2	Serosa	i.p.	1,10	18	10	3.0	38	20	21
	Pancreas	sq.	1	10	4	3.3	33	26	14
3	Subcutaneous	sq.	1,8	23	23	2.7	89	23	14
		i.m.	8	10	10	3.8	100	16	
4	Muscle	i.m.	15	10	10	4.0	33	14	8
5	"	i.m.	16	9	9	3.1	100	22	10
6-10	"	i.m.	11-24	49	49	4.3	97	19	9-10
11-19	"	i.m.	12-56	63	63	3.8	93	16	7-11
				RPL-17					
1	Liver	i.p.	1	19	14	3.4	47	56	15
2	"	i.p.	2	19	19	3.8	100	19	13
3	Liver	i.p.	1	25	25	4.4	100	16	10
	Kidney	i.p.	1	17	16	3.8	92	11	8
4	Liver	i.p.	2,9	27	23	4.9	77	24	12
		i.m.	9	9	6	3.8	57	17	—
5	Liver	i.p.	7,15	25	19	4.1	77	20	8
		i.m.	7	7	4	5.0	100	22	8
6-10	Liver	i.p.	15-26	64	52	4.1	84	17	10-11
	"	i.m.	23	3	3	2.7	100	15	—
	Blood cells	i.v.	15	11	6	4.8	50	13	11
11-16	Liver	i.p.	9-33	75	67	4.1	87	14	7-11
		i.m.	36	9	9	3.5	89	16	—
		sq.	33	5	4	4.0	67	12	—

i.p. = intraperitoneal. i.m. = intramuscular. sq. = subcutaneous.

record keeping, we have chosen to give them separate strain numbers.

The liver inoculum produced growths in 10 birds out of 19 inoculated, with an average survival of 22 days for those that died; however, passage was not attempted until the 27th and 36th day after inoculation. A high incidence of takes was obtained in the second passage by the intraperitoneal and subcutaneous routes. In the next passage the incidence increased to 100 per cent by either the subcutaneous or intramuscular route. This unanimity of takes by the intramuscular route was maintained in subsequent passages. Extensive metastasis to the viscera took place in all passages and the tumor mortality within 30 days was close to 100 per cent for all passages except the first, second, and fourth. The survival period decreased during the 19 passages from an average of 21 days from the first 5 passages to 16 days for the last 9 passages. This occurred in spite of the fact that older birds were used in the later passages.

TABLE III: SUMMARY OF INTRAMUSCULAR INOCULATIONS OF STRAINS RPL-14, -15, AND -16 (PASSAGES 2 TO 15 INCLUSIVE)

	RPL-14	RPL-15	RPL-16
Number inoculated	89	114	80
Per cent			
Negative	17	10	0
Primary tumor developed then regressed—bird survived	38	30	6
Primary tumor developed—bird died	9	10	1
Primary tumor developed—bird died with metastasis	36	50	93
Total positive	83	90	100

Strain RPL-17.—The liver of E1110R_3, which originated strain RPL-17, produced tumors within 120 days in 14 of 19 birds inoculated. Though the average survival time was 56 days, first passage was made in 15 days. The liver used for the next passage caused growths in all 19 birds inoculated, with an average survival period of 19 days. Subsequent passages with this strain were made with hepatic tumor suspensions injected intraperitoneally except in 1 instance (6th passage), where blood cells were used. These inoculations resulted in a high percentage of takes and high mortality. During the 16 passages there was very little change in the extent of visceral involvement, percentage dead in 30 days, or passage intervals; however, the survival period decreased to an average of 14 days for passages 11 to 16.

When kidney was compared with liver as a source of inoculum in the third passage similar results were obtained. Blood cells given intravenously in the 6th passage also produced similar mortality and similar pathological changes. The first intramuscular inoculation with this strain was made in the fourth passage, producing 6 takes out of 9 birds inoculated. Inoculation by the same route in the 16th passage produced intramuscular tumors in all 9 birds inoculated, with metastases in 8. Tumor suspension injected subcutaneously in the 11th passage produced local tumors and metastasis to the viscera of most birds injected.

Five cases of neurolymphomatosis developed in passages 4, 5, and 6 prior to 79 days of age. It is possible that they occurred from natural causes, inasmuch as the recipient chicks came from matings that in previous tests had been shown to produce chicks with a moderate percentage of neural lymphomatosis under controlled conditions. Thus it is probable that the neurolymphomatosis obtained was unrelated to the inoculation.

Gross lesions.—Neoplastic cells of strains RPL-14, -15, -16, or -17 injected into the pectoral muscle produced intramuscular tumors in 7 to 14 days (Figs. 1 and 3). The tumor usually increased the size of the pectoral muscle by 3 to 6 times in a period of 2 weeks. The growth was diffuse, yet compact, and in time extended throughout the length of both the deep and the superficial muscle. After a period of about 14 days, necrosis began to appear. If the bird survived the tumor receded, leaving a pectoral muscle that seemed normal upon gross and microscopic examination.

The tumor grew easily in the skin, since many nodules with a diameter of 1 to 2 cm. were noted at the point where the hypodermic needle pierced it during intramuscular inoculations. Such tumors would often ulcerate and recede if the bird survived.

Death of the inoculated birds was no doubt dependent upon visceral involvement, since very few died without gross lesions in one or more of the viscera. It has been assumed that the visceral involvement in birds inoculated in the pectoral muscle was due to metastasis.

Of the viscera, the liver was involved most frequently (Table IV). Usually it was enlarged to 3 times the normal size and the tumor areas were generally miliary in type and diffusely spread throughout the parenchyma (Figs. 2 and 6); such livers were invariably friable. In some cases, particularly in RPL-17 (Fig. 5), large gray tumors were separated by relatively normal liver tissue, whereas others had a combination of focal and diffuse tumor formation. The heart, when involved, usually showed separate nodules (Fig. 2). The testes, lungs, mesentery, and peritoneum showed either focal or diffuse involvement, whereas the kidneys, spleen, and ovary were diffusely involved as a rule. Except for slight differences in organ incidence between the 4 strains (Table IV) no differences of significance were detected upon gross examination.

Microscopic lesions.—Preliminary examination of the tissues of typical cases from each of the 4 RPL strains has shown definite similarities in their histological picture. The primary tumors and their metastatic foci contained mature lymphocytes, but the predominating cellular element was a less mature cell that had a definitely basophilic cytoplasm, a large nucleolus, and showed reactive variations in the nuclear chromatin. With some differences, the histological picture was similar to the tumors described by Pentimalli (15) and by Olson (13), and those observed in numerous cases of naturally occurring lymphomatosis (16).

Blood changes.—The blood changes after inoculation were similar in the 4 RPL strains. Unusual numbers of immature blood cells were found in circulation only during the intermediate and late stages of the disease, and often did not appear until a few hours before death.

Changes in the cellular constituents of the blood include the appearance of polychromatophil erythroblasts and basophil erythroblasts, an increase in the number of thrombocytes and the appearance of thromboblasts in varying number and degrees of maturation, the appearance of hemocytoblasts, usually an increase in the number of granulocytes, and a decrease in the number of lymphocytes; occasionally myelocytes have been observed.

Blood smears were not taken periodically on all birds inoculated; hence it cannot be said that all showed blood changes. However, in a limited examination involving strains RPL-14, -15, -16, and -17, blood smears were taken daily until death, and it was found that all birds showed one or more of the above changes at some time during the course of the disease, but usually in the terminal stages.

DISCUSSION

Under the conditions of these experiments, cellular inocula prepared from affected organs of certain birds with naturally occurring visceral lymphomatosis produced tumorous lesions in a relatively short time in a high proportion of birds inoculated, whereas similarly prepared material from other birds with visceral lymphomatosis elicited few or no lesions during the same period. There is some indication that these differences are attributable to the donor and not to the inoculum itself, inasmuch as in 3 cases where inocula were prepared from 2 tumorous organs of the same donor the 2 produced like results (Table I).

Four tumor strains were developed and transmitted in serial passage from 9 original inocula prepared from tumorous organs of birds with visceral lymphomatosis. A fifth strain, originating from a composite of lymphomatous nerve tissue, was maintained for 2 passages. Of the 9 birds in the second passage inoculated, 3 visceral tumor cases occurred in 32 days. Suitable donors were not obtained, hence no further passages could be made. It is not improbable that more strains similar to RPL-14, -15, -16, or -17 could have been developed from the original inoculations given in Table I if more strict surveillance of the inoculated birds throughout the entire experimental period had been maintained. The practice of using live birds as donors was strictly adhered to. The strains that were established came from the 4 inocula showing the greatest virulence at the first inoculation. Many chicks developed growths at an early age, giving ample opportunity for the selection of donors before death.

TABLE IV: GROSS INVOLVEMENT OF VISCERA IN BIRDS THAT DEVELOPED TUMORS AFTER INOCULATION WITH RPL-14, -15, -16, AND -17

Strain	Passage	No. of cases with visceral involvement	Percentage of cases										
			Adrenal	Gonad	Heart	Intestine	Kidney	Liver	Lung	Pancreas	Serosa	Proventriculus	Spleen
RPL-14	1-15	49	18	61	37	6	53	63	0	35	55	16	39
RPL-15	1-16	114	19	49	36	0	52	65	6	33	42	9	42
RPL-16	1-18	107	8	51	52	0	75	78	3	21	30	7	58
RPL-17	1-15	226	5	44	27	15	60	94	6	30	46	8	72

The characteristics of the 4 strains were similar, especially in the later passages. Tumors developed and deaths occurred in a relatively short time (1 to 4 weeks) in all strains. The extent and type of visceral involvement were much the same, except that in the first few passages by intraperitoneal inoculation RPL-14, -15, and -16 induced tumor growth locally and by direct extension in the visceral peritoneum. In contrast, few local tumors in the peritoneum were induced by RPL-17 when the same route of inoculation was used. Certain quantitative differences were demonstrated. A summary of data from birds that died following intramuscular inoculations in the second to 15th passages of strains RPL-14, -15, and -16 are given in Table III. Although there was but little difference in the total percentage of positives, RPL-16 had a much higher percentage of primary tumors with metastasis, a shorter survival period, and a smaller proportion surviving the inoculation than RPL-14, with RPL-15 midway between -14 and -16.

The data indicate that some increase in virulence

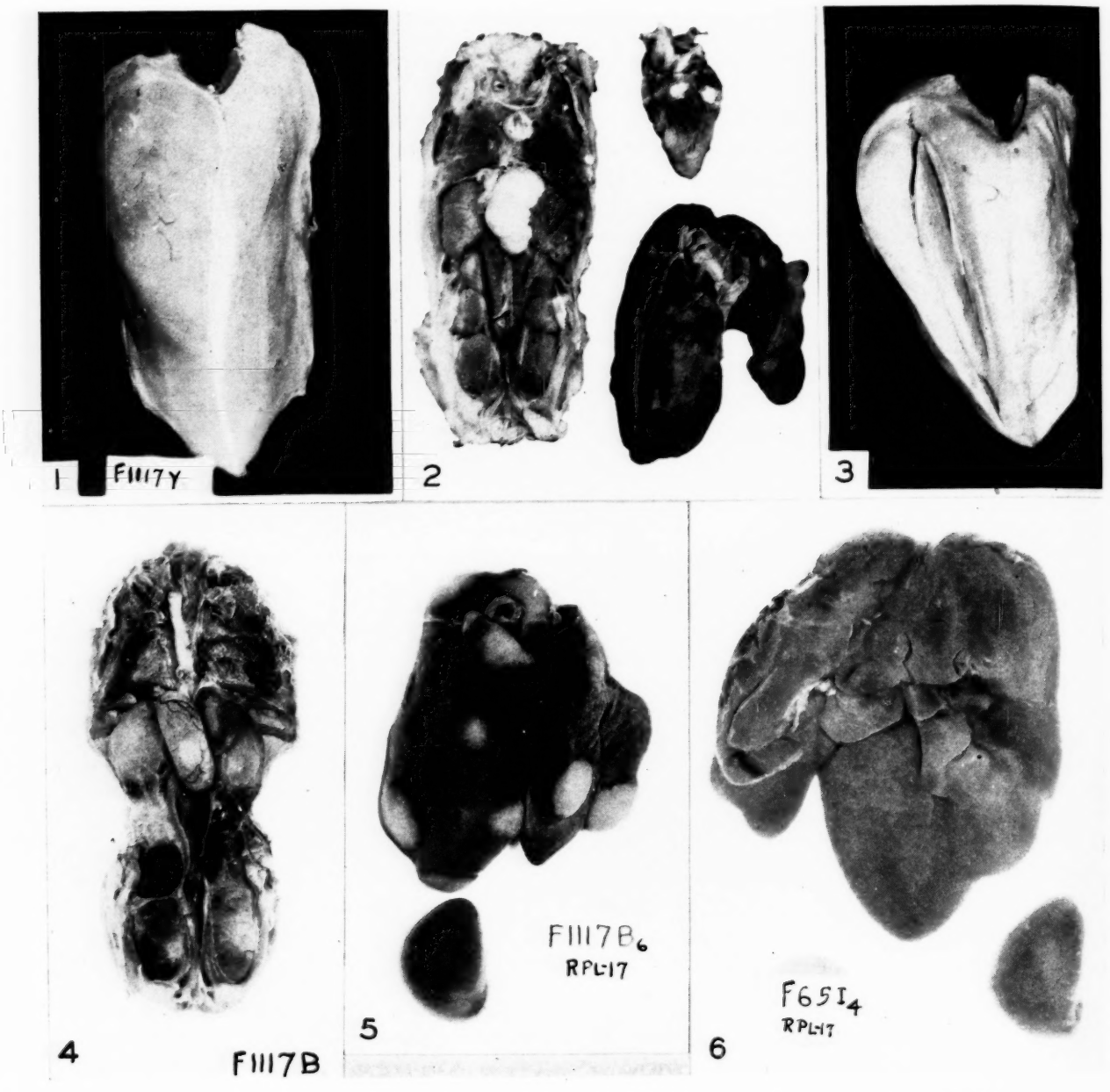

FIG. 1.—Pectoral tumor occurring 17 days after inoculation with RPL-16 tumor cell suspension from the 15th passage.

FIG. 2.—Diffuse involvement of the liver, kidney, lungs; the nodular areas in the heart; and the tumor of the left ovary resulting from the intramuscular inoculation of minced tumorous liver from an RPL-17 donor of the 18th passage.

FIG. 3.—Pectoral tumor from the case described in Fig. 2.

FIG. 4.—Extensive metastases to lungs, testes, kidneys, heart, and proventriculus observed 16 days after inoculation of RPL-17 liver tumor into the pectoral muscle.

FIG. 5.—Liver and spleen of bird described in Fig. 4. This liver illustrates the focal type of tumor. By contrast, the spleen is diffusely infiltrated.

FIG. 6.—Liver and spleen showing diffuse involvement in a bird that died 14 days after intraperitoneal inoculation with RPL-17 liver. Similarly involved were the kidney, lungs, and mesentery. Focal tumors occurred in the pancreas, intestinal wall, and peritoneum.

occurred in all strains during the several passages, which was most pronounced in RPL-16 and least in RPL-17. The percentage of takes in the intramuscular strains (RPL-14, -15, and -16) cannot be compared directly with that in the intraperitoneally inoculated (RPL-17), since in the former it is based on clinical examination of the breast muscle for the presence of a tumor, whereas in the latter it is based on necropsy findings; thus, birds of RPL-17 must die or be killed as donors to be available for examination. Many large intramuscular tumors, especially of RPL-14, and -15, have completely regressed. If intramuscular tumors can regress, there is no reason to believe that visceral tumors may not also regress, and the birds show no lesions at necropsy. These cases would then be recorded as negative, since visceral tumors cannot be identified by clinical examination; hence all positive cases will not be recorded when intraperitoneal inoculations are made.

A comparison of gross visceral involvement of the 4 strains for all passages and routes of inoculation is given in Table IV. In all strains the liver shows the highest percentage of involvement, varying from 63 per cent in strain RPL-14 to 94 per cent in RPL-17. The 5 organs showing the greatest frequency of involvement are, with 1 exception, the same in all 4 strains. The liver is followed by kidney, gonad, spleen, and the serosa. All strains except 14 showed lung involvement, whereas only RPL-14 and -17 had tumors in the intestinal wall. Other small differences are present; however, the visceral involvement obtained with the several strains was very similar in general.

The majority of cases in RPL-14, -15, and -16 were inoculated by the intramuscular route. Thus visceral involvement denotes metastases from the primary intramuscular tumor to the organs listed in Table IV, whereas most of the cases in RPL-17 were inoculated by the intraperitoneal route; therefore it is not possible to determine if some, or even any, metastasis took place. In spite of this difference in route of inoculation and in possible interpretation of visceral involvement, there is little difference in the number and frequency of organs involved in the cases that showed visceral lesions.

Similar transplantable tumors of the chicken have been described. Furth (3-5) developed a strain (Furth No. 2) from a case of naturally occurring visceral lymphomatosis. After several attempts he was able to transmit it by intramuscular passage, and obtained results with respect to takes and metastasis similar to the RPL strains, but different in that endotheliomas and myelomas were reported to have appeared occasionally in the inoculated birds. Pentimalli (15) obtained through serial passage a tumor that he designated a transplantable lymphosarcoma from a bird showing tumorous involvement of the viscera. Olson (12) developed a similar strain from a chicken with neoplasms of the liver, spleen, and other organs. Olson (12) stated: "The [Pentimalli's] original case from which the tumor was derived and the behavior of the tumor in experimental chickens was very similar to that obtained in the present work [Olson's]." Feldman and Olson (2), in discussing the origin of the Pentimalli and Olson tumors, state: "The original cases of both possessed features which would permit them to be classified as lymphocytoma and the principal reason for not doing so is that transplantability has not been demonstrated as a characteristic feature of lymphocytoma." The tumor described by Olson (12, 13) has been studied at this Laboratory in 89 serial transfers and does not differ essentially from the RPL strains -14, -15, and -16 in most of its pathological manifestations. The visceral tumors that Feldman and Olson call lymphocytomas are similar to, if not identical with, the condition known at this Laboratory (8), and by others (6, 7, 9), as visceral lymphomatosis.

The RPL strains described herein showed high malignancy in almost all the early inoculations, including the original. The second passage was made at intervals of 10 to 27 days. Metastasis was widespread and the average survival period very short. This is in contrast with the results of the early inoculations made by Olson (12), who experienced difficulty in obtaining suitable donors for passage and had to wait 99 days to make the second passage and 28 for the third. Pentimalli (15) obtained only 1 take in 4 birds inoculated in the second passage, and stated that "metastatic growths were seldom observed." Birds died with infiltration of the pectoral muscles in 4 to 8 weeks.

Olson (13) reported in the first 30 passages 14.4 per cent growth with metastasis and no primary tumors in 32.6 per cent of birds inoculated, whereas in the RPL strains metastasis occurred in 36 to 93 per cent (Table III) of all birds inoculated in passages 2 to 15, and the proportion that were negative ranged from 0 to 17 per cent. The differences in results of the first several passages between the Olson and RPL strains are even more notable than indicated, since the RPL data include only passages 2 to 15, and the negative cases and absence of metastasis occurred primarily in passages 2 to 5 inclusive.

Brewer (1) obtained several visceral tumor strains by intraperitoneal and subcutaneous inoculation of a cell suspension from birds with naturally occurring visceral lymphomatosis. Strain RPL-17 is very similar to Brewer's strains with respect to original donors, average survival time, mortality, and gross pathological lesions.

It appears that with the same route of inoculation

the pathological changes and transmission characteristics of the Pentimalli, the Olson, the Brewer, and the RPL strains are rather similar. Thus it is evident that tumors from cases of naturally occurring visceral lymphomatosis may, under favorable conditions, give rise to highly malignant transplantable strains. That this is not a rare phenomenon (14) is indicated by the fact that of the 6 original donors used to supply visceral tumors for the preparation of inocula, 3 produced a high incidence of tumors in a short time and only 1 gave entirely negative results.

It has further been demonstrated that highly malignant intramuscular and visceral strains can be obtained from cases of naturally occurring visceral lymphomatosis by transplanting tissue from selected donors to susceptible young chicks. Establishment and propagation of individual strains were accomplished by transferring material from the first birds of a particular inoculation that showed typical pathological alterations to healthy recipients.

Presumably many factors operate to influence the success of such inoculation. It is not the intent of this report to demonstrate them, but it may be suggested that such factors as genetic susceptibility to lymphomatosis, age of recipients, route of inoculation, and the malignancy of tissue transplanted probably played an important part in producing the virulent reactions.

The differences between the tumor strains described and naturally occurring lymphomatosis are rather striking in some respects. The propagated strains in all respects demonstrated, appear to have a much greater malignancy than naturally occurring lymphomatosis. However, the relation between the tumor of the original donor and those of the propagated strains must be fairly close, since the essential characteristics of each propagated strain were established within 1 or 2 passages, and within a relatively short period of time. The question of transmission by a filterable agent has not yet been investigated.

SUMMARY

Young chicks injected intraperitoneally with affected organs of chickens with visceral lymphomatosis developed lymphomatous tumors of the viscera in a relatively short time.

Inocula prepared from different donors varied greatly in their activity. The material showing the greatest potency induced tumors in 13 of the 17 birds inoculated and caused death in an average of 11 days after inoculation. Tissues that became involved included the abdominal wall, adrenal, gonad, heart, kidney, liver, mesentery, intestine, pancreas, peritoneum, proventriculus, and spleen. The least potent material, though grossly similar to the most potent, produced no tumors in 37 chicks injected during the experimental period of 86 days. Four inocula that elicited a high proportion of tumors were propagated by serial transfer through 15 to 19 passages made at intervals of 7 to 14 days. No essential differences in the gross pathology between the strains were noted.

The neoplastic cells, implanted in young chicks, induced lymphoid tumors that were both macroscopically and microscopically similar to the tumors providing the original inoculum.

The question of transmission by a filterable agent has not yet been investigated.

REFERENCES

1. Brewer, N. R. Personal Communication. 1944.
2. Feldman, W. H., and Olson, C., Jr. Neoplastic Diseases of the Chicken. *In* Diseases of Poultry. Edited by H. E. Biester. Ames: Iowa State College Press. 1943, Chapter 30, pp. 523-597.
3. Furth, J. Lymphomatosis, Myelomatosis, and Endothelioma of Chickens Caused by a Filterable Agent. II. Morphological Characteristics of the Endotheliomata Caused by this Agent. J. Exper. Med., **59**:501-517. 1934.
4. Furth, J. Lymphomatosis, Myelomatosis, and Endothelioma of Chickens Caused by a Filterable Agent. I. Transmission Experiments. J. Exper. Med., **58**:253-275. 1933.
5. Furth, J. Lymphomatosis in Relation to Fowl Paralysis. Arch. Path., **20**:379-428. 1935.
6. Johnson, E. P. Fowl Leukosis—Manifestations, Transmission, and Etiological Relationship of Various Forms. Va. Agric. Exper. Sta. Tech. Bull. 76, 1941.
7. Jungherr, E. The Avian Leukosis Complex. *In* Diseases of Poultry. Edited by H. E. Biester. Ames: Iowa State College Press. Chapter 18, pp. 367-414. 1943.
8. Jungherr, E. L., Doyle, L. P., and Johnson, C. P. Recommendations of the Pathology Committee. Tentative Nomenclature of the Avian Leukosis Complex. Second Collaborators' Conference at the Regional Poultry Research Laboratory, East Lansing, Mich. 1941, p. 119.
9. Lee, C. D., Wilcke, H. L., Murray, C., and Henderson, E. W. Fowl Leukosis. J. Infect. Dis, **61**:1-20. 1937.
10. Olson, C., Jr. Transmissible Fowl Leukosis. A Review of the Literature. Mass. Agric. Exper. Sta., Bull. 370, 1940.
11. Olson, C., Jr. A Simple Instrument for Mincing Tissue. Am. J. Vet. Res., **2**:295-296. 1941.
12. Olson, C., Jr. A Transmissible Lymphoid Tumor of the Chicken. Cancer Research, **1**:384-392. 1941.
13. Olson, C., Jr. The Serial Passage of an Avian Lymphoid Tumor of the Chicken. Cancer Research, **4**:707-712. 1944.
14. Olson, C., Jr. The Serial Passage of a Transmissible Lymphoid Tumor. Proc. 16th Annual Conference of Laboratory Workers in Pullorum Disease Control. 1944.
15. Pentimalli, F. Transplantable Lymphosarcoma of the Chicken. Cancer Research, **1**:69-70. 1941.
16. Prickett, C. O., and Burmester, B. R. Unpublished Data.
17. Waters, N. F. Breeding for Resistance and Susceptibility to Avian Lymphomatosis. Poultry Sci., **24**:259-269. 1945.

Abstracts

Reports of Research

Polycyclic Aromatic Hydrocarbons. XXXI. Some Nitrogenous Analogues of Chrysene, Pyrene and 3: 4-Benzphenanthrene. Cook, J. W., and Thomson, W. H. S. [Univ. of Glasgow, Glasgow, Scotland] *J. Chem. Soc.*, 395-399. 1945.

In view of the inhibitory action on tumor growth of some polycyclic compounds containing nitrogen atoms in the ring system, 6-azachrysene, 1-azapyrene, 5-methyl-4,6-diazachrysene, 3,4-benz-5-azaphenanthrene, and 3,4-benz-5,7-diazaphenanthrene were synthesized.—E. L. K.

Synthesis of the Differential Growth Inhibitor, δ-Hexenolactone. Haynes, L. J., and Jones, E. R. H. [Imperial Coll., London, England] *Nature, London,* **155**:730. 1945.

Medawar, Robinson, and Robinson (*Nature, London,* **151**:195. 1943) previously described the isolation, from a commercial malt extract, of small quantities of a steam-volatile substance that inhibited the growth of fibroblasts at concentrations that permitted the free growth of epithelial tissue. It seemed probable that the material was an unsaturated lactone, and it was found that synthetic specimens of *dl*-δ-$\Delta^{\alpha\beta}$-hexenolactone exhibited similar inhibitory properties. A new synthesis is described whereby this lactone can be obtained in about 35% overall yield from propylene oxide; the new method has the advantage of being readily adaptable to the preparation of related lactones.—A. H.

Inhibition of the Carcinogenic Action Produced by a Weakly Carcinogenic Hydrocarbon on a Highly Active Carcinogenic Hydrocarbon. Lacassagne, A., Buü-Hoi, and Rudall, G. [Radium Inst., Paris Univ., Paris, France] *Brit. J. Exper. Path.*, **26**:5-12. 1945.

Each series of mice was painted with a different mixture of 2 compounds consisting of a strong and a very feeble carcinogen (*i.e.*, methylcholanthrene + 1,2,5,6-dibenzfluorene; 1,2,5,6-dibenzanthracene + 1,2,5,6-dibenzacridine; methylcholanthrene + chrysene; methylcholanthrene + six methyl derivatives of benzacridine). Controls were painted with the strong carcinogen alone. Carcinogenicity of the solutions was assessed by noting the latent period of papilloma formation and of malignancy; lung adenomas, weight of spleen and liver, and body weight were recorded.

The authors are satisfied that addition of a feeble carcinogen inhibits the activity of a powerful carcinogen and explain this result by appealing to some such mechanism as competition between the 2 compounds for a substrate essential for cellular multiplication. The methyl acridines showed no inhibitory power, but no statement is made about their carcinogenicity.

The mechanism of the phenomenon of inhibition of carcinogenesis is discussed.—I. H.

Testing Carcinogen Analogues for Carcinogenic Activity. Dodds, E. C., Lawson, W., and Williams, P. C. [Courtauld Inst. of Biochem., Middlesex Hosp., London, England] *Cancer Research,* **5**:485-486. 1945.

Five compounds, whose structural relation to active carcinogens resembles that of stilbestrol to estrone, were tested for carcinogenic activity. Slight activity was demonstrated in one of them—α-ethyl-β-*sec*-butylstilbene—which is regarded as a benzpyrene analogue. The other compounds tested were: α,α,β-triphenyl-α-butylene, α,α-diphenyl-β-ethyl-α-amylene, α,α-diphenyl-β-ethyl-α-butylene, and 1,4-distyryl-benzene.—Authors' abstract.

The Effect of the Autoxidizability of Lipoidal Solvents on Sarcogenesis by 3,4-Benzpyrene. Rusch, H. P., Mueller, G. C., and Kline, B. E. [Univ. of Wisconsin Med. Sch., Madison, Wis.] *Cancer Research,* **5**:565-571. 1945.

The effect of various lipids on sarcogenesis, when used as solvents for 3,4-benzpyrene, was compared with certain chemical and physical characteristics of the lipids. The vehicles used were ethyl stearate, ethyl oleate, ethyl linolate, ethyl palmitate, methyl caprate, tricaprylin, lecithin, "mouse fat," corn oil, cottonseed oil (largely freed of antioxidants), "lard filtrate," and "lard residue." A pronounced retardation of tumor formation was observed with methyl caprate, ethyl linolate, lecithin, lard residue, and a petroleum ether extract of mouse carcasses.

With some lipids there was an indication that their effect on sarcogenesis could be correlated with their susceptibility to autoxidation *in vitro* and with the corresponding destruction of the dissolved hydrocarbon. Thus the addition of α-tocopherol to ethyl linolate, ethyl oleate, or cottonseed oil (largely freed of antioxidants) decreased the rate of destruction of 3,4-benzpyrene and favored the formation of tumors. However, tocopherol also enhanced the genesis of sarcomas when added to the saturated fatty acid ester, methyl caprate, a substance that is not susceptible to autoxidation. Furthermore, there was relatively little destruction of benzpyrene *in vitro* when it was dissolved in lard residue, yet this solvent had a striking inhibitory influence on the development of tumors.

No relationship was observed when the rate of disappearance of hydrocarbon from the tissues was compared to the incidence of tumor formation. Thus when 3,4-benzpyrene was dissolved in ethyl linolate or ethyl oleate (each with and without added tocopherol), lard residue, or lard filtrate and the mixtures were injected subcutaneously, the carcinogen disappeared at the same rate from the tissues of all the groups, but the final incidence of sarcomas varied from 3 to 77% depending on the solvent used.

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It seems evident from the results of these experiments that the variation in tumor formation observed when different lipids are used as solvents for a carcinogen cannot be attributed to any single characteristic of the oils. Instead, it appears that a variety of factors concerning the chemical and physical characteristics of the lipid, and the differences in tissue response to the solvent, all play an important role in carcinogenesis.—Authors' abstract.

Further Studies on Urethane-Induced Pulmonary Tumors. Henshaw, P. S., and Meyer, H. L. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**:415-417. 1945.

Previously, intraperitoneal injections of urethane had been found to induce lung tumors in A strain mice. The present investigation indicates that the subcutaneous implantation of crystalline urethane in 1 mgm. pellets and its oral administration as a 0.1% solution for drinking water are both effective means of inducing lung tumors at an early age in this strain of mice, and that the number of tumors per lung is increased by increasing the total dose of drug. Also, by giving urethane intraperitoneally at different periods during the life of the animals evidence was obtained indicating that most, though not all, of the lung nodules arise within 1 to 2 months after treatment, and that lung tissue that was not susceptible to tumor induction early may become so later on.—R. A. H.

Experimental Tumors in an Insect. Scharrer, B. [Sch. of Med., West. Reserve Univ., Cleveland, Ohio] *Science,* **102**:102. 1945.

In the insect *Leucophaea maderae* (Orthoptera) transection of the recurrent nerve resulted in the appearance of tumors in the organs normally innervated by this nerve. The tumors appeared within 10 days to several months after the operation and were found most frequently in the anterior portion of the midgut and in the salivary reservoir. In the foregut and salivary glands well developed tumors were relatively rare. Various types of control operations (allatectomy, castration, etc.) failed to produce tumors.—R. B.

The Physiological Effects of Sunlight on Man. Blum, H. F. [Nav. Med. Research Inst., Nat. Nav. Med. Center, Bethesda, Md.] *Physiol. Rev.,* **25**:483-530. 1945.

One section of this review summarizes the statistical, geographical, and experimental evidence, now considered quite conclusive, that sunlight is a major factor causing skin cancer in man. Experiments with animals have shown that the ultraviolet portion of the spectrum is responsible for the development of the tumors. The mechanism by which the irradiation produces the tumors is unknown. Various hypotheses have been put forward and are summarized by the author.—R. B.

Chronic Inflammation and Carcinogenesis. Woglom, W. H. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Cancer Research,* **5**:576-578. 1945.

Compared with the 3 causes so far known, the constant presence of a mild irritant (black silk thread) in the mamma of RIII virgin female mice was of no importance in determining the site or accelerating the initiation of mammary carcinoma.—Author's summary.

Effect of Change of Coat on the Growth of Epidermal Warts in Mice. Mottram, J. C. [Mt. Vernon Hosp., Northwood, Middlesex, England] *Nature, London,* **155**: 729-730. 1945.

It is well known that epidermal warts in mice, produced by painting with tumor-producing agents, often show periods when their growth rate is reduced or their size diminished, and they may even disappear temporarily. During observations on the growth of hair near warts produced by benzpyrene, it was noted that diminution in growth of the warts occurred, whereas in black mice the surrounding skin, normally pale, became pigmented as it does when the hair bulbs are enlarging preparatory to the formation of a new coat. Details are provided from which the conclusion was drawn that the growing hair bulbs cause a diversion of blood towards themselves and away from the overlying epidermis and warts; hence the blanching, the lowered growth rate, and the diminution in size of the warts.—A. H.

Influence of Age on Calcium in Epidermal Carcinogenesis Induced by Methylcholanthrene in Mice. Suntzeff, V., Carruthers, C., and Cowdry, E. V. [Barnard Free Skin and Cancer Hosp., and Washington Univ. Sch. of Med., St. Louis, Mo.] *Cancer Research,* **5**:572-575. 1945.

The role of calcium as a factor in the age difference seen in the response of epidermis of old and young mice of the New Buffalo and CBA strains of mice to methylcholanthrene was investigated. Old mice of both strains were found to contain more epidermal calcium than the young. The epidermis of both age groups of the New Buffalo strain responded similarly when treated with methylcholanthrene; that is, they underwent a nearly 50% decrease in the calcium content. However, the diminution in the calcium content of the CBA strain was less than that of the New Buffalo, and the young group showed about 50% less drop than did the old.—Authors' summary.

The Application of Sulfonamides to Gastrointestinal Disease. II. Gastric Excretion of Sulfonamides in Man. Schiff, L. [Univ. of Cincinnati Coll. of Med., and Cincinnati Gen. Hosp., Cincinnati, Ohio] *Gastroenterology,* **4**:3-4. 1945.

Patients with gastric carcinoma were among the subjects tested. The results have been published elsewhere (*J. Nat. Cancer Inst.,* **5**:369. 1945; abstr. in *Cancer Research,* **5**:604. 1945).—E. E. S.

Polarographic Response of Deproteinated Serum of Individual Rabbits Before and After Implantation with the Brown-Pearce Carcinoma. Westfall, B. B., Thompson, J. W., and Burk, D. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.,* **5**:407-414. 1945.

The proteose levels of rabbit sera were studied by determining the polarographic activity of sulfosalicylic acid filtrates. The activity was found to vary considerably in normal rabbits from animal to animal and in the same animal at different times. After intratesticular injection of normal-rabbit-testis mash, no change in serum proteose was noted. There was generally, however, a gradual increase in polarographic activity after intratesticular inoculation of the Brown-Pearce tumor. Although this increase usually did not occur until the tumors were palpable, the degree of increase could be correlated with

the size of the tumor only in extreme cases. Intercurrent respiratory infections also increased the activity of the filtrates. No qualitative differences were noted in the polarographic waves of the sera from normal and tumor-bearing animals.—R. A. H.

The Dynamics of Cell Respiration and Growth and the Immediate Cause of Cancer. Roder, F. [Brooklyn, N. Y.] *M. Rec.*, **157**:407-410; 474-476; 491-492. 1944.

A discussion.—E. E. S.

Changes in the Organs of Female C3H Mice Receiving Thiourea. Dalton, A. J., Morris, H. P., and Dubnik, C. S. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:451-454. 1945.

Thiourea was added to the diet of virgin C3H mice when they were 11 months of age. The animals were maintained on this regimen until they developed spontaneous mammary cancer, when they were killed and the ovaries, uteri, adrenals, and thyroids studied histologically. The thyroids showed the expected hyperplasia, red blood cells were found within their follicles, and a granular, greenish yellow pigment appeared in the cytoplasm in the distal portion of the follicular cells. No changes were seen in the uteri, but a general degeneration of the follicles and ova was noted in the ovaries, and there was a decrease in the osmiophilic material of the adrenal cortices.—R. A. H.

Influence of Nontoxic Amounts of Estradiol upon Carcinogenesis in Intact and Castrated Male and Female Mice of the Marsh-Buffalo Strain. Bischoff, F., Ingraham, L. P., and Clarke, G. J. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] *Cancer Research*, **5**:579-581. 1945.

In female mice of the Marsh-Buffalo strain, the maximum amount of estradiol that could be administered without producing toxic symptoms or significant losses in body weight was without effect on the development of mammary tumors in intact mice and without appreciable effect on the development of lymphoid tumors in both intact and castrated mice. Mammary tumors were produced in castrated female mice, but the incidence was less than that produced by toxic doses and less than that in intact controls. Lymphoid tumor formation was not significantly different in controls, whether intact male or female mice or castrated males. Lymphoid tumor formation was significantly increased by nontoxic dosage of estrogen in the castrated male. A comparison made upon litter mates castrated at 30 days of age and receiving identical treatment as to housing, food, and dosage with estradiol, revealed 23% development of tumors of the mammary gland in the castrated females against 3% in the castrated males.—Authors' abstract.

Influence of Breeding on the Incidence and Onset of Carcinoma of the Mammary Gland in Marsh-Buffalo Mice. Bischoff, F. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] *Cancer Research*, **5**:582-583. 1945.

In 48 mice of the Marsh-Buffalo strain, breeding was without influence upon the onset and accumulative incidence of cancer of the breast. This observation was in contrast to the experience of Marsh. The observations of Marsh in regard to tumor incidence in virgins were confirmed.—Author's abstract.

Estrus in Virgin Strain C3H (High-Tumor) and Virgin Strain A (Low-Tumor) Mice and in the Reciprocal (A × C3H) F_1 Hybrids. Deringer, M. K., Heston, W. E., and Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:403-405. 1945.

The estrus cycles of virgin C3H and A strain mice were followed for a period of 150 days in order to determine whether the difference in the incidence of mammary tumors in virgin females of these two strains was reflected in the estrus cycles. No differences in regularity or duration were found, but it was noted that the vaginas opened significantly later in the A strain than in the C3H strain or F_1 hybrids between A and C3H. The authors conclude that, "These findings contribute evidence that the difference between the strain C3H virgin females with a high tumor incidence and the strain A virgin females with a low tumor incidence, ... is manifested in part at least through the hormonal mechanism."—R. A. H.

Relation of the Milk Influence to the Carcinogenic Induction of Mammary Cancer in Mice. Kirschbaum, A., and Bittner, J. J. [Univ. of Minnesota Med. Sch., Minneapolis, Minn.] *Proc. Soc. Exper. Biol. & Med.*, **58**: 18-19. 1945.

The relationship of the carcinogen-induced to the spontaneous neoplasm can be studied in the case of mammary cancer by using the dba strain, which is susceptible to both types of tumor. The present experiments were designed to test the role of the milk influence in governing the response of the mammary gland to methylcholanthrene applied to the skin 2 to 3 times weekly as a 0.25% solution in benzene.

Strain dba males (sublines 12 and 212) were crossed with C3H females possessing the milk influence (Z stock) and with C3H females lacking the milk influence (ZB stock). Their hybrids were observed either as virgin or as forced-bred females during treatment with methylcholanthrene. Breeding females of the genetic constitution (ZB ♀ × dba ♂) × dba ♂ were also skin-painted with the carcinogen. These backcross animals lacked the milk influence but carried genetic susceptibility and hormonal influence.

Mammary cancer was induced in the absence of the milk influence. However, if breeding females, treated with methylcholanthrene, carried the milk influence, they developed mammary cancer earlier, in greater numbers, and with more rapid growth of the cancer than did genetically identical animals without this influence.—M. B.

Fate of the C3H Milk Influence in Mice of Strains C and C57 Black. Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:383-390. 1945.

Mice of the low mammary cancer strain C (1.4% in breeding females) were nursed by C3H foster mothers and then bred brother to sister for 11 generations without further foster nursing. Of the 20 foster-nursed C females, 70% developed mammary cancer, and in the subsequent generations the incidence of mammary tumors varied from 83 to 96%. (I × C3H) F_1 hybrid females fostered by these C mice also developed a high incidence of mammary

cancer. These data indicate that female mice of the low cancer strain C can transmit the milk influence through 11 successive generations or passages.

Two female mice of the low mammary cancer C57 black strain were also foster nursed by C3H females and then bred brother to sister. Neither of these females developed mammary cancer, but of 13 (I×C3H) F_1 hybrid mice foster nursed by them, 12 developed mammary cancer. One of 9 C57 black females of the so-called F_1 generation (from the inbred fostered females) but none of 11 hybrid mice fostered by them had mammary gland tumors. No mammary cancer developed in the 3 subsequent generations of C57 black females nor in genetically susceptible hybrid mice foster nursed by them.

This would indicate that, unlike the C strain, the C57 black strain is unable to propagate or transmit the milk influence through successive generations in sufficient amounts to incite cancer in susceptible hybrids. Apparently, then, the genetic constitution of inbred mice determines not only their susceptibility to the milk influence but also their ability to propagate or transmit it.—R. A. H.

Relation of Milk Influence to Mammary Tumors of Hybrid Mice. Andervont, H. B. [Nat. Cancer Inst., Bethesda, Md.] *J. Nat. Cancer Inst.*, **5**:391-395. 1945.

Female mice of the low cancer strain C were bred to male mice of the C3H strain. As each litter was born, the males were discarded and 1 to 3 young (I×C3H) F_1 hybrid females added to serve as test animals for the presence of the milk influence in the C mothers. The resulting (C×C3H) F_1 and fostered (I×C3H) F_1 females were bred at 2 months of age and each bore 3 litters in rapid succession, not being allowed to nurse the young. Fifty-two per cent of the (C×C3H) F_1 females developed mammary cancer as compared to 8% of the (I×C3H) F_1 mice nursed by the same C strain females. Extracts and extract concentrates were made from 11 of the (C×C3H) F_1 tumors and fed to 79 young "test" females none of which developed mammary tumors.

The author concludes that, "The results show that mammary tumors arose in hybrid mice derived from strain C females and strain C3H males and suggest but do not prove that a milk influence was not involved in the occurrence of the tumors."—R. A. H.

The Relative Importance of the Milk Agent and the Inherited Susceptibility to It in the Development of Mammary Tumors in Mice. Warner, S. G., Reinhard, M. C., and Goltz, H. L. [New York State Inst. for Study of Malig. Dis., Buffalo, N. Y.] *Cancer Research*, **5**:584-586. 1945.

The tumor rates of 4 groups of virgin mice, the Marsh albino (M) stock, the Bittner albino (A), and the reciprocal crosses of the 2 strains, were compared. The A ♀ × M ♂ hybrids showed a higher tumor rate (98.0%) than either of the parent stocks (A stock, 29.0% and M stock, 63.5%), and approximately double that of the reciprocal cross (53.0%). It was concluded that: (1) the extrachromosomal, or milk, agent is more concentrated in the A than in the M stock; (2) the M stock is genetically more susceptible to the milk agent than the A stock; and (3) the inherited susceptibilty of the physiological system is of greater importance than the milk agent in the development of mammary tumors in these 2 strains of mice.—Authors' abstract.

Apparition du cancer spontané chez deux souris (mère et fille) d'une lignée non cancéreuse (lignée XXX). [Appearance of Spontaneous Cancer in Two Mice (Mother and Daughter) from a Non-Cancerous Line (Line XXX).] Dobrovolskaia-Zavadskaia, N. *Compt. rend. Soc. de biol.*, **135**:721-724. 1941.

The 2 mice reported came from a line (XXX) in which no mammary tumor had been observed for 11 years.—G. H.

Genetic Aspects of the Cancer Problem. Blank, F. [New York, N. Y.] *Arch. Surg.*, **49**:301-315. 1944.

A general review of experimental and statistical investigations, with an extensive bibliography.—W. A. B.

Antigene und Krebs. VI. [Antigens and Cancer. VI.] Micheel, F., and Emde, H. [Münster Univ., Münster, Germany] *Ztschr. f. physiol. Chem.*, **280**:88-91. 1944.

Further experiments are reported on the effect of antigen treatment upon the incidence of tumors induced by a depot of 1 to 2 mgm. of benzpyrene in mice, and upon the growth of transplanted mouse tumors. In the animals treated with benzpyrene, the antigen treatment consisted either in cauterization of the skin of the mice by a drop of concentrated H_2SO_4 at the beginning of the carcinogenic treatment and repeated 4 weeks later, or in injections of pure serum albumin (horse). Mice with 76% tumor incidence after benzpyrene treatment showed only a 15% incidence after cauterization of the skin. Injection of 10γ of serum albumin into mice of the Swiss or dilute brown strain that received 2 mgm. of benzpyrene was without significant effect; however, a decrease in the rate of tumor incidence was observed in dilute brown mice that received only 1 mgm. of benzpyrene, indicating a correlation between dosage of the carcinogen and success of the antigen treatment. It is suggested that the concentrated H_2SO_4 contributes antigens by denaturing the tissue proteins or by favoring infection by antigen-producing bacteria.

Transplanted tumors (Ehrlich's adenocarcinoma and Lettré's ascites tumor) were not influenced even by injections of up to 50γ of serum albumin.—Z. D.

Antigene und Krebs. VII. Synthese von Verbindungen des Pektins mit Eiweissstoffen. [Antigens and Cancer. VII. Synthesis of Compounds Containing Pectin Coupled to Proteins.] Micheel, F., and Dörner, H. [Münster Univ., Münster, Germany] *Ztschr. f. physiol. Chem.*, **280**:92-99. 1944.

To find out whether the antigenic properties of artificial antigens depend on the presence either of a carbohydrate or of tyrosine or of both, 3 series of compounds were synthesized by coupling pectin azide directly to proteins (gelatin and globulin), or first to the tyrosine ethyl ester and the ethyl ester of glucosaminic acid and then to the protein molecule. Only the compounds containing both pectin and tyrosine proved to be antigenic (precipitin test). Their influence on tumors could not be tested, because of their high toxicity. The nonantigenic pectin-gelatin compounds were without influence on tumors (15γ injected twice a week during 28 to 30 weeks into mice bearing benzpyrene depots.)—Z. D.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

Heredity

Cerebral Glioma in Siblings. Riese, W., Meredith, J. M., and Zeass, I. S. [Med. Coll. of Virginia, Richmond, Va.] *South. M. J.*, **37**:424-428. 1944.

Two cases of glioma of the cerebral hemisphere, occurring in a brother and sister, ages 50 and 39 years respectively, are presented. Both terminated fatally, one postoperatively. Because of similarities between the 2 cases, the author feels that the familial association could scarcely be accounted for by coincidence.—W. A. B.

Radiation

Cancer Treatment with Radium Bearing Moulds. Wiener, K. [Milwaukee, Wis.] *Wisconsin M. J.*, **44**:297-300. 1945.

The chief use of radium-bearing molds (moulage) is in the treatment of cancers of the oral cavity, the lips, and less often of the face and external genitalia. Radium mold therapy has, as a rule, to be followed by deep x-ray therapy to the lymphatics of the neck, or, under certain conditions, by bloc dissection. Nine photographs are included.—M. E. H.

Cancer of the Larynx. A Radiotherapeutic Test as an Aid in Choosing Between Operation and Irradiation. Cutler, M. [Chicago Tumor Inst., Chicago, and Vet. Admin. Facility, Hines, Ill.] *Arch. Otolaryng.*, **39**:53-58. 1944.

A trial of external x-ray therapy by the author's "concentration method" is recommended in those cases of cancer of the larynx in which the patient is a poor surgical risk, or in which partial mobility of the structures indicates possible radiosensitivity. Twenty-five cases have been treated in this way.—W. A. B.

Chronic Massive Pericardial Effusion Following Roentgen Therapy for Carcinoma of the Breast. Blumenfeld, H., and Thomas, S. F. [Stanford Univ. Sch. of Med., and Dept. of Pub. Health of San Francisco, San Francisco, Calif.] *Radiology*, **44**:335-340. 1945.

A patient with carcinoma of the breast received preoperative radiation and, at the time of operation, about 3,000 r at a single dose to the open thorax. It was computed that the total tissue dose received by the pericardium was about 8,250 r during 9 days. After this treatment the patient developed progressive pericardial effusion and died 4½ years later. Autopsy showed pericardial effusion and marked thickening of the parietal pericardium. There was no evidence of residual neoplastic disease. Because of the danger of damage to the pericardium, massive irradiation of the open thorax with the dosage employed in this case is not recommended for the future.—R. E. S.

Skin and Subcutaneous Tissues

Benign and Malignant Moles (Nevi); Their Differentiation and Treatment. Montgomery, H. [Mayo Clin., Rochester, Minn.] *M. Clin. North America*, 968-977. 1944.

The author describes various types of benign and malignant nevi and the conditions with which they may be confused, and then discusses their treatment. "There is no uniformity of agreement regarding clinical or histopathologic classification or treatment of different types of nevi. . .

"Blue nevus, which is benign, has been confused frequently with melano-epithelioma. There is danger of malignant change following inadequate removal of benign pigmented nevi. Any nevus that is subject to repeated trauma and irritation or shows increased pigmentation to simulate melano-epithelioma should be radically removed. Hope for cure of melano-epithelioma is dependent on its early recognition and treatment, and complete removal before metastasis has occurred."—J. L. M.

The Surgical Treatment of Malignant Melanomas of the Skin. Webster, J. P., Stevenson, T. W., and Stout, A. P. [Presbyterian Hosp., and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *S. Clin. North America*, 319-339. 1944.

One hundred and sixty-two cases of malignant melanoma of the skin, recorded in the Laboratory of Surgical Pathology of the College of Physicians and Surgeons of Columbia University from 1905 through 1940, are reviewed with reference to the anatomical distribution, sex and age incidence, clinical and pathological features, differential diagnosis, and types of treatment.

"A large percentage of malignant lesions arise from moles that have been stimulated by the patient or by a physician. Complete eradication of all suspicious-looking lesions and malignant melanomas should be carried out. Irradiation is usually ineffectual. Radical surgery is preferable to cauterization. Depending upon the history and histologic examination of the lesion, radical regional lymph-node dissection should be performed."—J. L. M.

Carcinomatous Changes in Luetic Gumma of Skin. White, C. J. [Loyola Univ. Sch. of Med., Chicago, Ill.] *Urol. & Cutan. Rev.*, **49**:52-53. 1945.

The author states he has seen 3 instances of malignant change in a luetic gumma of the glabrous skin, and describes in detail one in which the lesion was located on the shoulder.—V. F. M.

Nervous System

Papilledema in Optic Neuritis and Tumor of the Brain. Hammes, E. M., Jr. [Mayo Clin., Rochester, Minn.] *M. Clin. North America*, 957-967. 1944.

This report is based on 25 cases of brain tumor in which choked disk was present, and on 25 cases of optic neuritis in which there was elevation of the head of the optic nerve.

Criteria are proposed for distinguishing whether papilledema that is demonstrable ophthalmoscopically and is not associated with pallor of the optic disk is due to increased intracranial pressure or to optic neuritis.—J. L. M.

Intraspinal Lipomas. Report of Cases; Review of the Literature, and Clinical and Pathologic Study. Ehni, G., and Love, J. G. [Mayo Clin., Rochester, Minn.] *Arch. Neurol. & Psychiat.*, **53**:1-28. 1945.

A report of 7 cases and an analysis of 42 from the literature.—M. E. H.

Ear

Cholesteatoma with Fistula into the Labyrinth. Von Leden, H., and Williams, H. L. [Mayo Clin., Rochester, Minn.] *Arch. Otolaryng.*, **39**:432-433. 1944.

A case report.—W. A. B.

Adenoma of the Ceruminous Glands. Adler, H. J., and Sommer, I. [Chicago Eye and Ear Coll., Chicago, Ill.] *Arch. Otolaryng.*, **39**:533-535. 1944.

A case report and review of the literature. Only 2 of the previously recorded cases were proved.—W. A. B.

Female Genital Tract

Meigs' Syndrome in a Case of Multilocular Pseudomucinous Cystadenoma of the Ovary. Millett, J., and Shell, J. [Hempstead, N. Y., and Freeport, N. Y.] *Am. J. M. Sc.*, **209**:327-335. 1945.

Case report and discussion.—J. G. K.

Ovarian Fibromas and Theca Cell Tumors. Report of 78 Cases with Special Reference to Production of Ascites and Hydrothorax (Meigs' Syndrome). Rubin, I. C., Novak, J., and Squire, J. J. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Obst. & Gynec.*, **48**:601-616. 1944.

Ascites was noted in 7 of 23 cases of theca-cell tumor, and to a small extent was also present in 1 of 2 cases of papillary fibroadenoma and in 2 of 30 cases of fibroma. Of 23 earlier cases (fibroma and theca-cell tumor taken together), 9 showed ascites. Hydrothorax was present in only 2 of the 78 patients. Attempts to evaluate experimentally, in rabbits, rats, and mice, the factors responsible for the production of Meigs' syndrome were inconclusive.—A. K.

Cellular Diagnosis of Uterine Cancer by Centrifuge. Ayre, J. E., and Dakin, E. [Roy. Victoria Hosp., McGill Univ., Montreal, Canada] *Canad. M. A. J.*, **53**:63-66. 1945.

The centrifuge cytology test for uterine cancer is performed by immersing cervical secretions in a fixative solution of ether and 95% alcohol, centrifuging, and embedding the sediment in paraffin blocks, sections of which are examined microscopically. For staining the slides, ordinary hematoxylin and eosin are generally satisfactory, but Papanicolaou's stain gives greater nuclear clarity and definition. The test has proved correct in over 90% of 28 cases studied. The malignant cells are usually clustered together and therefore are more readily detected than in slides made directly from cervical or vaginal smears. The findings in a typical case of squamous carcinoma of the cervix are presented, and comparison is made of malignant cells in (a) the cervical smear, (b) the tissue biopsy, and (c) the centrifuge cytology test.—M. E. H.

Surgery of Uterine Cancer. Bainbridge, W. S. [New York, N. Y.] *M. Rec.*, **158**:161-163. 1945.

A variety of therapeutic measures, advocated in the past for cancer, are mentioned in the development of the thesis that surgical removal is still the best treatment. Radium is said to have a very limited scope, being chiefly useful in the treatment of skin tumors. Even in seemingly hopeless inoperable cases of uterine cancer, surgery is said to have been of considerable benefit. There is an extensive discussion of the purposes of the author's combined procedure entailing arterial ligation and lymphatic block prior to excision; such treatment in 62 cases of advanced cancer was followed by no operative mortality, and 18 patients lived 6 to 33 years afterward.—E. E. S.

Carcinoma of the Uterine Fundus with Metastasis to the Brain. Report of a Case. Hodge, G. B., and Steelman, H. F. [Duke Univ. Hosp. and Sch. of Med., Durham, N. C.] *Arch. Neurol. & Psychiat.*, **53**:218-221. 1945.

In the case reported both the primary and the secondary sites were examined grossly and microscopically during life.—M. E. H.

Pelvic Lymphadenectomy in the Treatment of Cervical Cancer. Morton, D. G. [Univ. of California Med. Sch., San Francisco, Calif.] *Am. J. Obst. & Gynec.*, **49**:19-31. 1945.

Preoperative treatment with x-rays may make pelvic lymphadenectomy unnecessary in the surgical treatment of cervical cancer. The incidence of lymph node involvement was 11.4% in 35 cases in which operation was carried out after x-radiation, as compared with 39.3% in 28 cases in which there was no preoperative roentgen therapy, despite the fact that the former group included many more advanced cases. However, lymphadenectomy apparently saved the lives, and led to 5 year cures, of at least 3 women.—A. K.

The Periodic Gynecological Examination. Thoms, H. [Yale Univ. Sch. of Med., New Haven, Conn.] *Connecticut M. J.*, **9**:185-187. 1945.

A plan is suggested for the follow-up clinics of obstetrical and gynecological wards to extend their service to include a periodic gynecological and general physical examination. Statistics are quoted to show the great value of such a program for the early recognition and cure of many varieties of pelvic disease including cancer.—M. E. H.

Male Genital Tract

Some Observations on the Pathological Anatomy of Benign Prostatic Enlargement. Swyer, G. I. M. [Oxford Univ., Oxford, England] *J. Path. & Bact.*, **56**:365-375. 1944.

From a detailed study of serial sections of prostates, the author has demonstrated that connections may exist between the utricular and prostatic glands of normal prostates. In his view, the earliest changes of prostatic hypertrophy consist of glandular hyperplasia occurring anterolaterally to the urethra in the caudal half of the prostate, namely in those zones of the prostate that show

the highest growth potential as evidenced by the longest persistence of active growth. The nature of intravesical projection of the enlarged prostate and the relationships of the uterus masculinus are discussed. Photomicrographs and drawings are included.—L. W. P.

Incidence of Prostatic Calculi in Association with Benign Hyperplasia or Malignant Lesions of the Prostate Gland. Cristol, D. S., and Emmett, J. L. [Mayo Clin., Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **19**:265-267. 1944.

"This study is based on 686 cases of malignant disease of the prostate gland and 4,136 cases of benign prostatic hypertrophy in which transurethral resection was performed at the Clinic in the 5 years, from 1938 to 1942, inclusive. In all of these cases roentgenographic examination of the prostatic regions was performed. Excluded from these figures are those cases in which transurethral resection was performed for the removal of calculi that were not associated with benign hyperplasia or malignant lesions, or cases in which satisfactory roentgenographic visualization of the prostatic region was not obtained."

Endogenous prostatic calculi were present in 288 (7%) of the cases of benign hypertrophy and in 43 (6%) of the cases of malignant lesions. In 72% of the latter 43 cases the grade of the malignancy was 1 or 2. Thus this study of a large series of patients is in disagreement with the commonly accepted dictum that prostatic calculi are rarely found in cases of carcinoma of the prostate gland. It suggests that the incidence of prostatic calculi in cases of benign hyperplasia and in cases of malignant disease of the prostate is practically identical. Any grade of malignancy may be associated with prostatic calculi.—J. L. M.

Oral Cavity and Upper Respiratory Tract

An Unusual Case of Osteoma of the Nasal Accessory Sinuses. Hanley, J. S. [New York, N. Y.] *Laryngoscope*, **54**:235-237. 1944.

Report of a case of 3 distinct and separate osteomas of the nasal sinuses in which the patient's presenting symptom was enophthalmos. Two of the masses were ivory-like and 1 was cancellous.—W. A. B.

A Mixed Tumor of the Nasal Septum. Ersner, M. T., and Saltzman, M. [Temple Univ. Sch. of Med., Philadelphia, Pa.] *Laryngoscope*, **54**:287-296. 1944.

A report of a case in which the encapsulated tumor was completely removed surgically and no irradiation used.—W. A. B.

Cancer of the Lip. Freeman, D. B. [Moline, Ill.] *Illinois M. J.*, **87**:94-96. 1945.

A general discussion. Prognosis in these cases is dependent on the time when treatment is instituted and on the thoroughness of the eradication of tumor tissue the first time treatment is given.—M. E. H.

Vascular Polyp of the Vocal Cord. Myerson, M. C. [New York, N. Y.] *Arch. Otolaryng.*, **39**:250-258. 1944.

Hoarseness, vocal fatigue, and sensation of a foreign body are the symptoms of a vascular polyp of the vocal cords, and these are severe enough to warrant surgical removal in all cases. The author doubts that the untreated polyp ever becomes malignant.—W. A. B.

Laryngectomy for Laryngeal Cancer. McCall, J. W., and Stover, W. G. [St. Luke's Hosp., and Huron Road Hosp., Cleveland, Ohio] *Laryngoscope*, **54**:659-676. 1944.

Forty-five cases in which laryngectomy was performed for laryngeal cancer are presented. Twenty-four patients received speech training preoperatively, and all but 4 developed a good esophageal voice. The authors recommend complete laryngectomy for both intrinsic and extrinsic cancer of the larynx, and cite the survival of 31 patients in this series.—W. A. B.

Solitary Neurofibroma of the Larynx. Smith, T. T. [Jefferson Hosp., Philadelphia, Pa.] *Arch. Otolaryng.*, **39**:144-151. 1944.

Three cases of neurofibroma in this rare site are reported, 2 in boys 6 years of age and the third in a 26 year old male.—W. A. B.

Chondroma of the Larynx. McCall, J. W., Dupertuis, S. M., and Gardiner, F. S. [St. Luke's Hosp., Cleveland, Ohio, and Pittsburgh Eye and Ear Hosp., Pittsburgh, Pa.] *Laryngoscope*, **54**:1-17. 1944.

A report of 2 cases and review of the literature, with a tabulation of 30 cases recorded since 1925. Treatment is always surgical removal of the tumor; when the growth is very extensive it requires laryngectomy.—W. A. B.

Paralysis of the Larynx. Fox, J. R. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Arch. Surg.*, **49**:388-389. 1944.

Six cases are reported in each of which the patient developed hoarseness as the first sign of metastasis from 14 months to 12 years after radical mastectomy for carcinoma. In all these cases laryngoscopy indicated paralysis of one or both recurrent laryngeal nerves, due to metastatic carcinoma of the chain of lymph nodes surrounding them.—W. A. B.

Plummer-Vinson Syndrome with a Post-Cricoid Web. Ball, K. P. (for Beaumont, G. E.) *Proc. Roy. Soc. Med.*, **38**:134. 1945.

A woman aged 52, taking a deficient diet without meat, had dysphagia, cracks and ulcer about the mouth, koilonychia, and a postcricoid web; the hemoglobin level was 45%. Under iron treatment the stomatitis and koilonychia disappeared, and the hemoglobin rose to 99%.—E. L. K.

Intrathoracic Tumors—Lungs—Pleura

Adenoma of the Trachea and the Bronchus. Schwartz, L. [New York, N. Y.] *Arch. Otolaryng.*, **39**:231-242. 1944.

Two cases of adenoma of the bronchus and one case of adenoma of the trachea are presented. These tumors show a marked tendency to recur despite bronchoscopic removal and irradiation. In the present cases, one patient was apparently cured by removal of the tumor and electrocoagulation of its base, and the other 2 patients developed recurrences.—W. A. B.

Hemoptysis. Etiology, Pathogenesis and Treatment. Adams, R., and Ficarra, B. J. [Lahey Clin., Boston, Mass.] *Lahey Clin. Bull.*, **4**:110-115. 1945.

The pathologic processes resulting in pulmonary hemorrhage vary according to the underlying etiology. Persistent daily hemoptysis, yielding small amounts of blood,

over a period of a few weeks, is almost surely caused by carcinoma. However, in only 58% of proved cases of carcinoma of the lung is hemoptysis a symptom. Adenoma of the bronchus is seen with a tenth the frequency of cancer, and blood-streaking at intervals is one of the typical symptoms of this condition. Attention is called to the long average duration of blood-streaked sputum (2 years) often occurring in patients with bronchial adenoma before they are seen for diagnosis and treatment. Diagnostically this period represents a long time lag, but prognostically it reflects the relative nonmalignancy of bronchial adenoma in comparison with bronchogenic carcinoma.—M. E. H.

Bronchogenic Carcinoma. Ainhum. Two Interesting Case Reports. Otsuka, M. [Chicago, Ill.] Report to Chicago Roentgen Society, Nov. 9, 1944. From abstr. in *Proc. Inst. Med. Chicago,* **15**:284-285. 1945.

The case of bronchogenic carcinoma in a 32 year old man was unusual in that the lesions were multiple. The clinical features were those of pulmonary tuberculosis.—M. E. H.

The Differentiation of Bronchiogenic Carcinoma and Pulmonary Tuberculosis. Pillsbury, N. R., and Wassersug, J. D. [Norfolk Co. Hosp., South Braintree, Mass.] *New England J. Med.,* **232**:276-278. 1945.

A study of 12 proved instances of bronchogenic carcinoma in patients who were admitted to a tuberculosis hospital. Only 2 of these patients had tuberculosis. The clinical and roentgenologic similarities between the two diseases are pointed out, and the importance of prompt diagnostic bronchoscopy is emphasized.—C. W.

Intracranial Metastasis of Carcinoma of the Lung. Tinney, W. S., and Moersch, H. J. [Mayo Clin., Rochester, Minn.] *Arch. Otolaryng.,* **39**:243-244. 1944.

The first symptoms of bronchogenic carcinoma may be those of increased intracranial pressure, resulting from metastasis to the brain. Of 448 patients with carcinoma of the lung at the Mayo Clinic, 52 had symptoms referable to the central nervous system. A thorough general examination, including roentgenograms of the thorax, should be performed in every case in which an intracranial or intraspinal lesion is suspected.—W. A. B.

Intrathoracic Mediastinal Lipoma. Wiper, T. B., and Miller, J. M. *Am. J. Surg.,* **66**:90-96. 1944.

A case of an unusually situated large lipoma in the anterior mediastinum is reported. Air injection into the pleural space to provide a contrast medium in x-ray examination was used as an aid in delineating the extent of the tumor. The tumor was extirpated, and the patient made an excellent recovery.—W. A. B.

Gastrointestinal Tract

Pernicious Anemia and Carcinoma of the Stomach —Autopsy Studies Concerning Their Interrelationship. Kaplan, H. S., and Rigler, L. G. [Univ. of Minnesota, Minneapolis, Minn.] *Am. J. M. Sc.,* **209**:339-348. 1945.

Autopsies on 23,231 persons 45 years of age or older revealed carcinoma of the stomach in 36 of the 293 cases of pernicious anemia found. The incidence of gastric cancer was more than 3 times as great in the persons with pernicious anemia as in the remaining autopsy population of the same age.—J. G. K.

Simultaneous Primary Carcinomas of the Stomach and Sigmoid. Pemberton, J. DeJ., and Seefeld, P. H. [Mayo Clin., Rochester, Minn.] *Am. J. Surg.,* **66**:393-395. 1944.

A case is reported in which both primary tumors (an adenocarcinomatous ulcer of the stomach and adenocarcinoma of the sigmoid) were diagnosed before operation in a 68 year old man who had had symptoms for 3 months.—W. A. B.

Sarcoma of the Small Intestines. Bodenheimer, J. M. [T. E. Schumpert Memorial Sanitarium, Shreveport, La.] *Am. J. Surg.,* **66**:404-406. 1944.

Report of a case.—W. A. B.

Carcinoma of the Colon. Cole, W. H. [Univ. of Illinois Coll. of Med., Illinois Research Hosp., and Educational Hosp., Chicago, Ill.] *Rocky Mountain M. J.,* **42**:169-178. 1945.

A study was made of 50 consecutive cases of carcinoma of the colon, observed in the Illinois Research Hospital during the past few years. Partial or complete obstruction occurred in 60% of patients with tumors on the left side and in 16.6% of patients with tumors on the right. The incidence of vomiting was apparently not related to obstruction since vomiting was present in about the same proportion of patients in the groups with tumors on the left and right (19.7 and 25%, respectively). The mortality rate in the entire series was 10%, and the rate was lower in the group with tumors on the left than in that with tumors on the right. Age also influenced the mortality rate, which was 20% in patients 65 years or more as compared with 6.9% in patients under 65. Improvement in the prophylaxis of infection, by penicillin and sulfonamides, will justify more frequent use of one stage resections with primary anastomosis.—M. E. H.

Chronic Ulcerative Colitis Followed by Carcinoma: Report of Twenty-Six Cases. Sauer, W. G., and Bargen, J. A. [Mayo Clin., Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **19**:311-316. 1944.

The purpose of this paper is to bring up to date the series of cases started in 1928 by one of the authors (J. A. B.). The 17 cases in the early report, together with the 7 presented in 1935 and the 26 described herein, make a total of 50 cases of carcinoma complicating chronic ulcerative colitis reported from the Clinic. A brief summary of the clinical and pathologic findings in this latest group of 26 cases is presented in tabular form.—J. L. M.

Carcinoma and Lymphosarcoma of the Colon. Tilton, B. T. [Lincoln and Manhattan State Hosps., New York, N. Y.] *Am. J. Surg.,* **66**:300-308. 1944.

The author compares carcinoma and lymphosarcoma of the colon from the standpoints of site of predilection, course and prognosis, and therapy. A case of lymphosarcoma of the descending colon in a woman 73 years of age is presented.—W. A. B.

Combined Intercolic and External Fistula Caused by Carcinoma of the Sigmoid. Gratiot, J. H., and Nunes, A. J. [Monterey Co. Hosp., Monterey, Calif.] *Am. J. Surg.,* **66**:265-268. 1944.

In this case the initial operation was performed because of acute pain and a mass in the left inguinal region that

proved to be an abscess. Further examinations, after a fecal fistula had occurred, showed obstruction in the sigmoid. At the second operation, a carcinoma of the sigmoid was found to have ulcerated into the mid-colon and into the abdomen with resultant abscess formation.—W. A. B.

Errors in the Diagnosis of Neoplastic Lesions of the Rectum, Rectosigmoid and Colon. KIRSNER, J. B., and PALMER, W. L. [Univ. of Chicago, Chicago, Ill.] *M. Clin. North America,* 278-285. 1944.

The seven case reports presented emphasize the fact that the early diagnosis of carcinoma of the colon and rectum depends upon a thorough investigation of all symptoms regardless of how trivial they may appear. The investigation must include a detailed history of the patient's illness, a complete physical examination, digital examination of the rectum, adequate proctosigmoidoscopy, careful x-ray study of the colon, a blood count, and analysis of the stools for occult blood.—J. L. M.

Carcinoma of the Rectum. WOODWARD, N. W. [Oklahoma City, Okla.] *J. Oklahoma M. A.,* **38**:89-93. 1945.

The author attempts resection whenever it is possible to remove the growth, even if there is little or no hope of cure. Reference is made to 31 cases in which operation was performed at University Hospital. Symptoms, signs, diagnostic features, and operative technic are discussed.—E. E. S.

Carcinoma of Rectum and Anus. MILLER, G. I. [Brooklyn, N. Y.] *M. Rec.,* **158**:166. 1945.

A brief summary of the known character and behavior of these tumors.—E. E. S.

MESENTERY

Dermoid Cysts of the Mesentery. JAMES, C. F., JR. *Am. J. Surg.,* **65**:116-119. 1944.

A report of a case in which the diagnosis was suggested preoperatively by x-ray examination, and a review of the literature.—W. A. B.

MULTIPLE TUMORS

Occurrence and Significance of Multiple Primary Cancers. WU, S. D. [Chicago, Ill.] Report to Chicago Path. Soc., Nov. 13, 1944. From abstr. in *Proc. Inst. Med. Chicago,* **15**:281. 1945.

Necropsy records of University of Chicago Clinics from 1923 to 1943 inclusive revealed a 3.7% incidence of multiple primary cancers among the 816 cases of cancer. The average age of individuals with multiple primary cancers was found to be higher than that of individuals with single cancers. Age and susceptibility are suggested as factors in multiple carcinogenesis.—M. E. H.

Multiple Primary Malignant Lesions. LICHTMAN, A. L. [Mayo Foundation, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.,* **19**:317-323. 1944.

A case is reported of 3 primary malignant lesions of the colon, which developed in the course of 17 years, with successful resection of each lesion. The author discusses his reasons for believing that each of the lesions (colloid adenocarcinoma of the cecum, adenocarcinoma of the descending colon, and colloid adenocarcinoma of the sigmoid) was a primary one.

In a second case, coincident primary adenocarcinomas of the rectum and lung were removed.

"It is important to evaluate apparently metastatic and recurrent malignant lesions with an eye toward the possibility of their being separate primary lesions."—J. L. M.

MISCELLANEOUS

A propos de quelques tumeurs malignes observées au Soudan français. [Some Malignant Tumors Observed in the French Sudan.] BABLET, J., and SICÉ, A. *Bull. Assoc. franç. p. l'étude du cancer,* **29**:77-87. 1940.

Three cases of malignant tumor (adenocarcinoma of the sigmoid, sarcoma of the scalp, and atypical adenocarcinoma of the parotid) in natives of the French Sudan are reported.—G. H.

CANCER CONTROL AND PUBLIC HEALTH

A Step in Cancer Control. A Review of the First 600 Cases Examined by the Cancer Prevention Clinic. WEBSTER, A., STENHOUSE, E., PARSONS, E., PHILLIPS, A., STETLER, P., OLIVER, M., AUSTIN, M., CONNELLY, M., ORTMAYER, M., and CUSHMAN, B. [Chicago, Ill.] *Illinois M. J.,* **87**:119-126. 1945.

A report of the first year's work. No treatments were given and no biopsies were done, patients being referred to their private physicians for such services. Eight patients had skin lesions that were clinically carcinoma; 5 patients had suspected melanoma, and wide surgical excision with biopsy was recommended. Nineteen patients were advised to have breast biopsies; 18 complied and 8 proved to have carcinoma. Two recurrences of breast tumor were found. Seven patients were advised to have cervical biopsies; no positive reports have been received from this group. Twelve patients with bleeding not associated with the menses were advised to consult their doctors; 1 proved to have adenocarcinoma of the uterine fundus, another, inoperable pelvic disease. One patient had a friable mass in the rectal ampulla. Numerous pathological conditions were found that were in no way related to cancer. The number of positive malignant growths is probably greater than reported because not all the private physicians have filed their findings with the clinic.—M. E. H.

Considérations biologiques sur le cancer du brai. [Biologic Considerations on Tar Cancer.] FIRKET, J., and MALTER, A. [Service provincial du Ministère du Travail et de la Prévoyance sociale, et l'Université de Liège, Liège, Belgium] *Bull. Assoc. franç. p. l'étude du cancer,* **29**:24-35. 1940.

Among 126 laborers, from 20 to 60 years of age or more, working in a tar factory in Liége, the incidence of cancer was no higher than in the general population. The authors conclude that the general hygienic precautions employed in the factory prevented the development of cancerous lesions of the skin. They suggest that lung tumors did not occur because either (1) the tar particles present were too large to penetrate the alveoli, or (2) the carcinogenic properties of tar for human beings may be relatively slight.—G. H.

Book Review

WHAT IS LIFE? THE PHYSICAL ASPECT OF THE LIVING CELL. By Erwin Schrödinger. Cambridge: University Press. 1944. viii + 91 pages.

This short and absorbing work is based upon a course of public lectures delivered at Trinity College, Dublin, about two years ago, under the auspices of the Dublin Institute for Advanced Studies. As the reaction on the part of a theoretical physicist of extreme distinction to his contact with biological data and conceptions, the book is of the first importance for workers in every department of biology: they will probably be few who, having assimilated its conclusions, remain other than profoundly and lastingly influenced. The book is a symptom —and its author recognizes it to be such—of the increasing need for a synthetic approach to fact and theory. The need is perhaps nowhere greater than in the domain of research in cancer, and what follows here is in part a summary, however inadequate, which will have achieved its purpose if it acts merely to direct more general attention to the relevance of the subject for a special field. In cancer research, as in every other sphere, we need primarily facts and yet more facts; and it matters nothing if their discovery is born of planned investigation or the play of accident and chance. But given the facts we require also the power of ideas and the compelling force of synthesis, if our labors are to add to the growing and changing *structure* of knowledge, and not produce a mere inchoate jumble of observations in unintelligible assortment. However, anyone attempting the synthesis must obviously do so within the inevitable restrictions imposed by his own equipment and experience: ". . . . it has become next to impossible for a single mind fully to command more than a small specialized portion" of the whole field of science. Our author modestly acknowledges his own limitations in biology, although it will surely be recognized, and with admiration, that he has triumphed over them to a quite remarkable degree. But Schrödinger has the immense advantage of being relatively untrammelled by genetic jargon (and the state of mind that this induces), and his success in delineating the hereditary mechanism (Chapter II) seems to the writer to be due to this as much as to any other factor. Mathematical deduction is scarcely used at all in the treatment; not, be it noted, that the subject is sufficiently simple to be explained without it, or that the mathematics is dispensed with in a semipopular account, but rather that the material is much too involved to be accessible to mathematical methods.

At the outset, Schrödinger poses the main question, to illumine which is the purpose of the book: "How can the events which take place within the spatial boundary of a living organism be accounted for by physics and chemistry?"; and he gives a preliminary answer: That the obvious inability of contemporary physics and chemistry to account for such events is no reason at all for doubting that they can so be explained. The reply is at once carried very much farther, in the statement that this inability of present-day physics and chemistry is amply accounted for, so that it is possible to say precisely *why* they could not conceivably explain what happens within a living organism: The structure of the vital parts of living organisms differs entirely, in its statistical relations, from that of the matter with which the chemist and the physicist ordinarily deal.

Schrödinger starts his pursuit through another question: "Why are the atoms so small?", and soon transposes it to read, "Why must our bodies be so large compared with the atom?" They are so because in common with other large-scale physical systems they must exhibit a certain degree of physical orderliness; that is, must obey strict physical laws to a required degree of accuracy. This organisms could not do, were they composed of a moderate number of atoms only and sensitive to the impact of a single atom, "because we know all atoms to perform all the time a completely disorderly heat motion, which, so to speak, opposes itself to their orderly behaviour and does not allow the events that happen between a small number of atoms to enrol themselves according to any recognizable laws. Only in the cooperation of an enormously large number of atoms do statistical laws begin to operate and control the behaviour of these *assemblées* with an accuracy increasing as the number of atoms involved increases. It is in that way that the events acquire truly ordered features."

Such precision as is based on the large numbers of participating atoms Schrödinger illustrates by three examples. First, paramagnetism, in which an observed weak magnetization is the outcome of two rival tendencies; namely, the magnetic field, which aims at constraining all the molecules parallel to one another, and the heat motion, which makes for random orientation. Secondly, the law of diffusion, the exactitude of which is due only to the enormous numbers of molecules that co-operate. And thirdly, the limits of accuracy of measuring with the torsion balance, represented by a light body suspended by a long thin fiber in equilibrium orientation. "In choosing lighter and lighter bodies and thinner and longer fibres—to make the balance susceptible to weaker and weaker forces—the limit was reached when the suspended body became noticeably susceptible to the impacts of the heat motion of the surrounding molecules and began to perform an incessant, irregular 'dance' about its equilibrium position. . . . . Though this behaviour sets no absolute limit to the accuracy of measurements obtained with the balance, it sets a practical one. The uncontrollable effect of the heat motion competes with the effect of the force to be measured and makes the single deflection observed insignificant. You have to multiply observations, in order to eliminate the effect of the Brownian movement of your instrument."

Schrödinger concludes his account of the classical physi-

cist's approach to the nature of life, by a reference to the $\sqrt{n}$ rule: The laws of physics are inaccurate within a probable relative error of the order of $1/\sqrt{n}$, where n is the number of molecules co-operating to bring about that law. "You see from this again that an organism must have a comparatively gross structure in order to enjoy the benefit of fairly accurate laws, both for its internal life and for its interplay with the external world. For otherwise the number of co-operating particles would be too small, the 'law' too inaccurate. The particularly exigent demand is the square root. For though a million is a reasonably large number, an accuracy of just 1 in 1000 is not overwhelmingly good, if a thing claims the dignity of being a 'Law of Nature.'"

The account above, which Schrödinger would call the classical physicist's expectation and which by some might be thought to have an air of the obvious, he now shows, far from being trivial or platitudinous, to be wrong. We now know that incredibly small groups of atoms, much too small to display exact statistical laws, do in fact play a dominating role in the orderly events within a living organism, having control of the observable large-scale features and functions that the organism acquires in the course of development. From an unembroidered account of the mechanism of heredity in mitosis, meiosis, fertilization, and crossing-over, Schrödinger passes to consider the maximum size of a gene as a cube of side 300A. Accepting this estimate, which to a large extent must be the weakest part of any such argument, Schrödinger then draws attention to the fact that since 300A is only about 100 or 150 atomic distances in a liquid or a solid, and a gene can therefore contain not more than about a few million atoms, the atoms are too few (from the $\sqrt{n}$ rule) to assure sufficient precision in any hereditary mechanism, where the degree of permanence of the transmitted characters is of a far higher order.

Consideration of the high degree of permanence and *durability* of the gene material brings us to its mutability; especially mutations in the sense of de Vries and of Bateson, and, one may add, somatic mutations, of which the cancer cell may be an example, always discontinuous but not necessarily considerable in the extent of the jump. Schrödinger suggests such facts would remind the physicist, in a figurative sense, of quantum theory, and he then shows that the likeness is much more than superficial, and that the mutations may in fact be due to quantum jumps in the gene molecule.

The high degree of permanence of the gene material has a corollary in the comparative rarity of mutation, but the so-called natural rate can be increased to a high multiple by irradiation with x-rays or gamma rays, the mutations produced differing in no way save their number from those that occur spontaneously. And there is an energy relation: "The experiments on X-ray-produced mutations give the impression that every particular 'transition,' say from the normal individual to a particular mutant, or conversely, has its individual 'X-ray coefficient,' indicating the percentage of the offspring which turns out to have mutated in that particular way, when a unit dosage of X-ray has been applied to the parents, before the offspring was engendered." Schrödinger then recapitulates the laws governing the induced mutation rate: That the increase is exactly proportional to the dosage of radiation, and that the coefficient of increase remains unaffected by wave length employed provided the same dosage, in r units, is applied. Mutation appears to be due to a single event, represented by an ionization or similar process, occurring within some critical volume of the cell. For the size of this "target," which has to be hit for a mutation to occur, Schrödinger follows M. Delbrück (in *Nachr. a. d. Biol. d. Ges. d. Wiss. Göttingen,* 1:189. 1935), where this author arrives at a size of only about 10 average atomic distances cubed, comprising therefore 1,000 atoms. If the accepted "size of the gene" is to be reduced to these dimensions, how are its lawfulness and permanence to be reconciled with statistical and classical physics? In Schrödinger's view the explanation is supplied by quantum theory, which therefore gives a basis for the hereditary mechanism. The poet has expressed what must constantly recur to the scientist: "Strange that a harp of a thousand strings, should keep in tune so long"; and here at least is a part of the answer:

"The great revelation of quantum theory was that features of discreteness were discovered in the Book of Nature, in a context in which anything other than continuity seemed to be absurd according to the views held till then." Thus while a body on the large scale changes its energy continuously, a sufficiently small system is found by its very nature to possess only certain discrete amounts of energy, called its peculiar energy levels, and the event of transition from one state to another is called a quantum jump. (In all these matters Schrödinger adopts the version usually given in popular treatment, without reference to occasional indeterminateness; it is of scientific interest that he regards it as sufficient for this special purpose. But he confesses to "the bad conscience of one who perpetuates a convenient error"). If the second state or configuration has the greater energy ('is a higher level'), the system must be supplied from outside with at least the difference of the two energies to make the transition possible. To a lower level it may change spontaneously, emitting the surplus of energy in radiation. Among the states that may be assumed by a given selection of atoms there may be a lowest level (implying a close approach of the nuclei to each other), in which the atoms form a molecule. Here again the molecule possesses a certain stability, and cannot change unless at least the necessary energy difference (a well-defined quantity) be supplied from outside.

Of obvious and supreme biological importance is the stability of molecules as affected by temperature. A molecule at the absolute zero of temperature would be regarded as the system of atoms in its state of lowest energy, and a definite supply of energy is required to raise it to the next higher state. "Considering the entire irregularity of heat motion, there is no sharp temperature limit at which the 'lift' will be brought about with certainty and immediately. Rather, at any temperature (different from absolute zero) there is a certain smaller or greater chance for the lift to occur, the chance increasing of course with the temperature. . . . ." This "time of expectation" for the lift to occur depends on the ratio of two energies, one

being the energy difference (W) required to effect the lift, the other (kT) characterizing the intensity of the heat motion at the temperature in question (T): the chance for effecting the lift (after Polanyi and Wigner) is smaller, and the time of expectation longer, the higher the lift itself compared with the average heat energy; *i.e.,* the greater the ratio W:kT, and small variations in the ratio produce enormous changes in the expectancy. Schrödinger quotes examples in which, for a ratio of 30, the time of expectation might be as short as 0.1 sec., and would rise to 16 months and 30,000 years for ratios of 50 and 60 respectively; but $W = 30kT$ is already extremely rare.

In most of these cases it is assumed that the quantum jump effects either disintegration or a different configuration of the same atoms; that is, an isomeric molecule. But this conception requires to be amended for the cases in which free passage between two levels may be obstructed, quite apart from the energy supply, even from a higher to a lower level. There are no spontaneous transitions from one state towards another, when the two configurations are not neighboring, and transition can take place only over intervening configurations that have a greater energy than either of them. Most significant, such isomeric transitions are those of the greatest import for biology: "Transitions with no threshold interposed between the initial and the final state are entirely uninteresting, and that not only in our biological application. They have actually nothing to contribute to the chemical stability of the molecule. . . . . They have no lasting effect, they remain unnoticed. For, when they occur, they are almost immediately followed by a relapse into the initial state, since nothing prevents their return."

These relations at once recall the familiar and similar distinctions between fluctuating and permanent biological modifications, between variants that are irreversible and those that return to the parent form with greater or lesser ease. And the manner in which the subject is presented cannot fail to make it of prime interest to every biologist who has to do with the transformation of one kind of cell into another, and with the relative stability of different cellular types. So far as the study of cancer itself is concerned, we must inquire what proportion of the observed incidence (however small) may be regarded as spontaneous natural mutation, due to chance fluctuations of the heat motion, and contrariwise, what is the expectancy (again however slight) of a tendency to spontaneous regression or reversion? While these considerations may seem remote by everyday standards, it is only by such means that we can reach an understanding in the most thorough and completely fundamental sense, and utilize to the utmost the data acquired by other methods and by experiment. So far as one can judge, the emergence of the cancer cell is frequently the result of a process reminiscent of "directed" mutation, and the malignant cell is chiefly characterized by a high degree of autonomy and permanence, so that reversion—assuming it to be possible—must be of the utmost rarity. But the last few years have brought increasing recognition that the autonomy of the cancer cell, although of a high order, is not absolute, and more recently we have seen the beginnings of the application of this principle in chemotherapy, albeit in the most favorable circumstances and on a limited scale. The general approach that Schrödinger has indicated should inspire us eventually to seek some comprehension of the energetics of cellular transformation; the heat or other radiation emitted by a cell in passing from one level of organization to another, and the energy required to facilitate its return. In this way we may hope to attain a measure of the cell stability, and estimate the physical feasibility of its reversion to the original state.

If any note of criticism is to be offered, it is that the book does not sufficiently convey the *growth* of these ideas in their relation to biology. Many of them, of course, are not entirely new, or even recent, and some similar analogies of a simpler kind have long been used; *e.g.,* that genes behave in a manner similar to tautomeric forms in equilibrium, that a tautomeric structure of protoplasm may explain the alterations occurring in the genetic elements by means of mutation, and that the changes produced in the cell may be likened to the shift of a polygon of forces. But even if much of what Schrödinger has to tell us has often been thought, it can only rarely, if ever, have been so well or succintly expressed. In some fields, although clearly not in all, it must be confessed that the impact of physics on biology has been disappointing in its results, perhaps because its use was over-sanguine, or premature, so that the history of biochemistry and the history of biophysics have inevitably been utterly different. But here we have a highly valid application of physics, on its theoretical side, direct to the plane of physiology and cell behavior. From Delbrück's picture of the hereditary substance it emerges that living matter, while not eluding the established laws of physics, involves other laws hitherto unknown. In Schrödinger's opinion we must be prepared to find living matter working in a manner that cannot be reduced to the ordinary laws of physics; not on the ground that there is any "new force" directing the behavior of the single atoms, but because the construction is different from anything yet tested in the physical laboratory. The new principle is a genuinely physical one: nothing else than the principle of quantum theory over again.

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